Structure-based Design of a Bromodomain and Extraterminal Domain (BET) Inhibitor Selective for the N-terminal Bromodomains that Retains an Anti-inflammatory and Anti-proliferative Phenotype

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ABSTRACT The bromodomain and extraterminal domain (BET) family of epigenetic regulators comprises four proteins (BRD2, BRD3, BRD4, BRDT) each containing tandem bromodomains. To date, small molecule inhibitors of these proteins typically bind all eight bromodomains of the family with similar affinity resulting in a diverse range of biological effects. To enable further understanding of the broad phenotype characteristic of pan-BET inhibition, development of

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inhibitors selective for individual, or sets of, bromodomains within the family is required. In this regard, we report the discovery of a potent probe molecule possessing up to 150-fold selectivity for the N-terminal bromodomains (BD1s) over the C-terminal bromodomains (BD2s) of the BETs. Guided by structural information, a specific amino acid difference between BD1 and BD2 domains was targeted for selective interaction with chemical functionality appended to the previously developed I-BET151 scaffold. Data presented herein demonstrates that selective inhibition of BD1 domains is sufficient to drive anti-inflammatory and anti-proliferative effects.

INTRODUCTION Bromodomains are a diverse family of protein interaction modules that bind acetylated lysine (KAc) post-translation modifications (PTMs) on histone tails and function as regulators of transcriptional control.[1] The human genome encodes at least 46 bromodomain-containing proteins (BCPs) encompassing 61 unique bromodomains, including the bromodomain and extraterminal domain (BET) family (BRD2, BRD3, BRD4 and BRDT).[2] The bromodomains within the BET family have been targeted with small molecules which have been used to probe the function of the BET proteins in a variety of disease settings including oncology, inflammation, infectious disease, and metabolic disorders.[3] Each BET family member contains tandem bromodomain modules and most BET inhibitors published to date bind all eight bromodomains with similar affinity i.e. pan-inhibition. Despite the promising disease-modifying effects of pan-BET inhibitors, greater insight into how individual bromodomains contribute to particular biological responses can be realized with domain-selective inhibitors and there have been recent advances in this area.

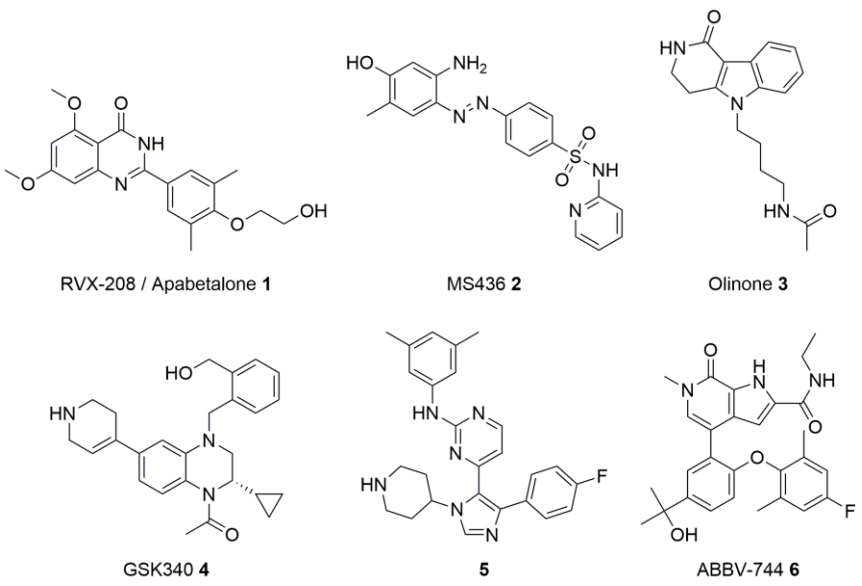


Figure 1. Structures of domain-selective BET inhibitors.

The emergence of domain-selective BET inhibitors (Figure 1)[4-11] such as RVX-208/Apabetalone (**1**) have provided further biological understanding. This clinical candidate originated from a HepG2 Apo-A1 upregulation assay and was subsequently found to inhibit the BET bromodomains with preferential binding to the C-terminal bromodomains (BD2s) over the N-terminal bromodomains (BD1s).[4, 5] The degree of selectivity was dependent on the method of measurement, but based on dissociation constants from isothermal titration calorimetry (ITC) Picaud et al. reported BD2 selectivities of 23-fold and 8-fold for BRD2 and BRD4, respectively.[5] Independent ITC measurements by McLure and coworkers also determined preferential binding to BD2 domains but with an increased magnitude (82-fold for BRD2 and 29-fold for BRD4).[6] Gene-expression studies with RVX-208 showed that selective inhibition of BD2 only modestly affected BET-mediated transcription and suggested a dominant role of BD1 in transcriptional control.[5]

While optimizing the selectivity of a diazobenzene CREBBP inhibitor towards BRD4 bromodomains, Zhang et al. discovered MS436 (**2**, Figure 1), a molecule possessing approximately

10-fold selectivity for BRD4 BD1 over BRD4 BD2 but with negligible domain selectivity in BRD3.[7] Cellular studies with MS436 demonstrated inhibition of the BRD4 function in gene transcription through blockage of both NF-κB-directed nitric oxide production and interleukin (IL)-6 cytokine expression in LPS-stimulated macrophages.

Another report described the structure-based optimization of a CBP hit compound into Olinone (**3**, Figure 1), an inhibitor selective for BET BD1 domains.[8] At the concentrations tested, either no, or weak (>300 μM) binding of Olinone to the BD2 domains of BRD2, 3 and 4 was detected in FA and ITC assays, respectively. However, Olinone possessed only modest affinities (K_i = 17.2–39.5 μM by FA, and K_d = 3.3–8.6 μM by ITC) for BD1 domains. Studies with Olinone demonstrated its ability to promote oligodendrocyte progenitor differentiation whereas a pan-BET inhibitor suppressed this response.

A publication from Law et al. described the discovery of a series of BET BD2-selective tetrahydroquinoxalines exemplified by GSK340 (**4**, Figure 1).[9] The potency of GSK340 for BD2 domains ranged 316–50 nM in mutant FRET assays with 8–50-fold selectivities over BD1 domains. Potencies and selectivities were confirmed by surface plasmon resonance (SPR) and BROMOscan[12] assays. A number of BD2-selective tetrahydroquinoxalines inhibited release of the proinflammatory cytokine MCP-1 in LPS-stimulated human PBMCs; an effect also observed for GSK340 in human whole blood. Collectively, these data indicated selective inhibition of BD2 domains was sufficient to drive immuno-inflammatory effects.

Divakaran et al. recently reported the development of the dual kinase-BET inhibitor (**5**, Figure 1).[10] Several 1,4,5-trisubstituted imidazoles displayed preferential binding to the BD1 domains of BET and IC_{50} values for **5** at BRD4 BD1 and BRD4 BD2 were determined as 1.8 μM and >100 μM, respectively; the K_d for p38α was 0.47 nM. A reduction of the oncogene c-Myc in MM.1S

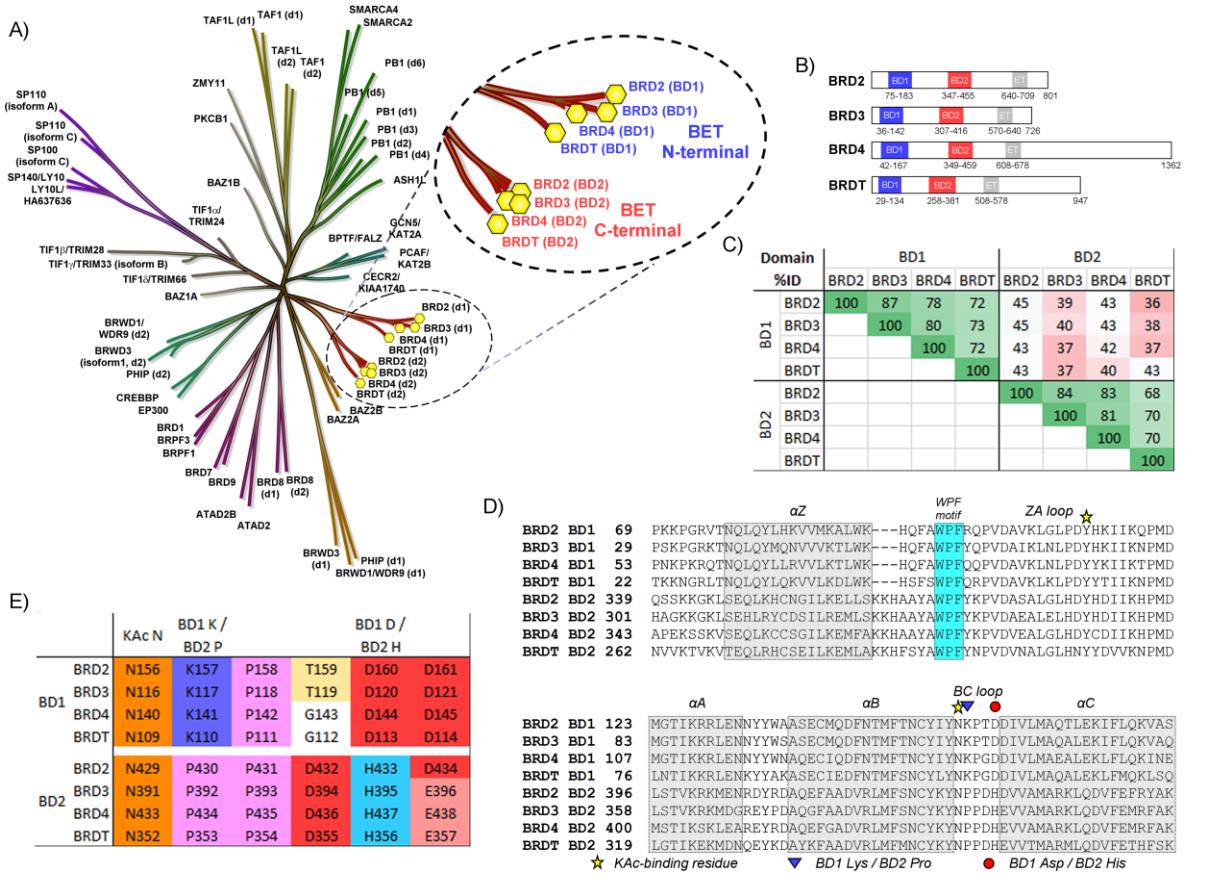
cells, a hallmark of BET inhibition, was observed upon treatment with **5**, while anti-inflammatory effects in A549 cells, including reduced TNFα-induced transcription and inhibition of IL-8 cytokine production, were observed. A subsequent publication from these researchers demonstrated effective ablation of p38α activity from triazole-based BET inhibitors while increasing BRD4 BD1 activity, however, domain selectivity was diminished.[13]

The BD2-selective BET inhibitor ABBV-744 (**6**, Figure 1) was very recently reported.[11, 14] This inhibitor possessed high potency for the BD2 domains and greater than 290-fold selectivity over the BD1 domains of BRD2, 3 and 4 in FRET assays.[11] The high potency and selectivity for BRD4 BD2 over BRD4 BD1 was recapitulated in SPR and NanoBRET assays. Testing in 59 cancer cell lines revealed that the antiproliferative effects of ABBV-744 were narrowed compared to a pan-BET inhibitor. In vivo assessment of ABBV-744 demonstrated efficacy in a prostate cancer xenograft model and reduced side effects highlighting the therapeutic potential for selective inhibition of BET BD2 domains.

Studies with these small molecules have provided initial insight into the function of the BD1 and BD2 domains. However, the modest domain-selectivities or potencies displayed by most of these inhibitors limit their usefulness for fully investigating domain-selective inhibition. For example, contributory effects towards the biological response relating to engagement of lower affinity bromodomains cannot be ruled out for modestly-selective inhibitors. Additionally, the high concentrations required for weak inhibitors to fully engage the intended bromodomain(s) risk off-target effects. While other methods such as protein deletion,[15-17] site-directed mutagenesis[18] and a "bump-and-hole" approach[19] have contributed to our understanding, the development of inhibitors demonstrating significant domain selectivity and potency is crucial to elucidate the specific roles of BD1 and BD2 domains in native protein environments.

Accordingly, we herein report the structure-based design and development of a probe molecule exhibiting both high affinity and selectivity for BD1 domains of the BET family. Studies with this molecule revealed anti-inflammatory and anti-proliferative activities comparable to pan-BET inhibitors.

RESULTS AND DISCUSSION



		KAc N	BD1 K / BD2 P			BD1 D / BD2 H	
BD1	BRD2	N156	K157	P158	T159	D160	D161
	BRD3	N116	K117	P118	T119	D120	D121
	BRD4	N140	K141	P142	G143	D144	D145
	BRDT	N109	K110	P111	G112	D113	D114
BD2	BRD2	N429	P430	P431	D432	H433	D434
	BRD3	N391	P392	P393	D394	H395	E396
	BRD4	N433	P434	P435	D436	H437	E438
	BRDT	N352	P353	P354	D355	H356	E357

Figure 2. (A) Bromodomain phylogenetic tree (adapted from Chung et al.[20]) with enlarged section showing the BET BD1 and BD2 bromodomains. (B) Domain architecture of the BET proteins. (C) Percentage sequence identity between the eight BET bromodomains (>70% colored green and ≤40% in red). (D) Sequence alignment of BET bromodomains. (E) BET family BC loop residues colored by amino acid type.

The four BET isoforms each contain two bromodomains (BD1 and BD2), all highly conserved with respect to the rest of the bromodomain phylogenetic tree (Figure 2A and 2B). The BD1 and BD2 domains form two distinct sub-branches of the tree, with higher conservation (68–87% identity) between bromodomains across isoforms (e.g. BRD2 BD1 versus BRD4 BD1, Figure 2C) and lower conservation (36–45% identity) between the two bromodomains within each isoform (e.g. BRD2 BD1 and BRD2 BD2).[21] One key position close to the KAc binding site differing between BD1 and BD2 domains is found in the BC loop (Figure 2D and 2E). The BD1 domains of all isoforms possess an aspartic acid at one position (BRD2 Asp160, BRD3 Asp120, BRD4 Asp144, and BRDT Asp113), whereas the BD2 domains contain a histidine (BRD2 His433, BRD3 His395, BRD4 His437, and BRDT His356). Two crystal structures of the BRD4 BD1 and BD2 domains (PDB codes 3zyu[22] and 2yem[21]) are representative of published high-resolution X-ray structures and show the structural consequences of this substitution (Figure 3A). Although the backbones of Asp144 and His437 align closely, their sidechains adopt different rotameric states, an observation confirmed in most BRD2 and BRD4 X-ray structures published to date (Figure 3B). In BRD4 BD1, Asp144 preferentially adopts the *gauche*(+) chi1 rotamer and points away from the KAc binding site ("out"). In contrast, the BRD4 BD2 His437 lies *trans* and points towards it ("in").

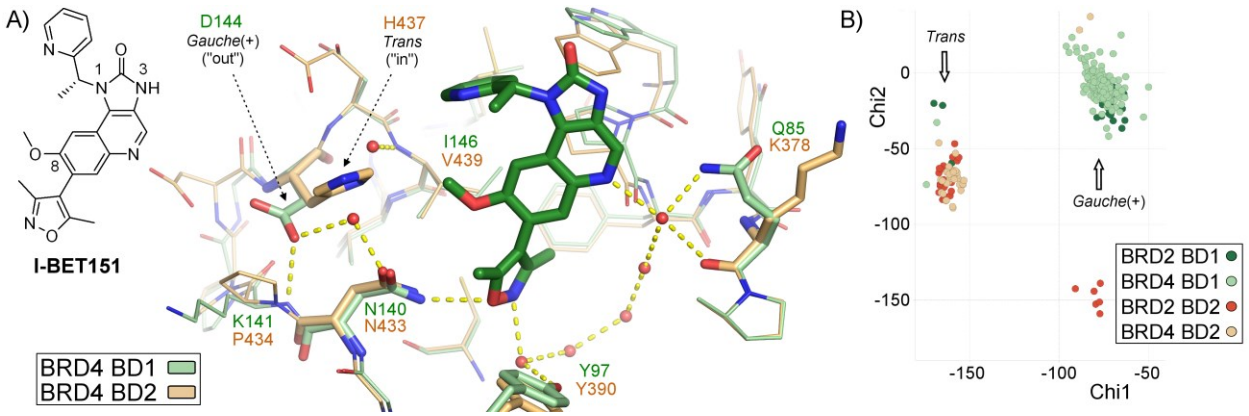


Figure 3. (A) Complex of imidazoquinolinone I-BET151 (**7**) in BRD4 BD1 (carbon = green, PDB 3zyu[22]) superimposed on BRD4 BD2 protein (carbon = orange, PDB 2yem[21]). (B) Distribution of Asp/His chi1 torsion angles of BRD2 and BRD4 BD1 and BD2 domain X-ray structures in the protein databank.

A possible explanation for this different preference lies in the hydrogen-bonding interaction between Asp144 and the backbone NH of Lys141 in BRD4 BD1. Lys141 is conserved as lysine in all BET BD1 domains, whereas in all BD2 domains it is replaced by proline (Pro434 in BRD4). Not only does Pro434 lack the hydrogen-bonding ability of a backbone NH, it might also clash with His437 in its *gauche*(+) conformation, so in BRD4 BD2 His437 points towards the KAc site. In doing so it makes a direct hydrogen-bond with the backbone NH of Val439, the capping residue of the C-helix. In BRD4 BD1 there is no internal hydrogen-bond to Ile146 (the equivalent residue of BD2 Val439). Instead, the C-helix of BRD4 BD1 is capped by a water molecule. An equivalent water molecule has previously been identified as highly conserved in numerous fragment complexes of BRD2 BD1.[20] Using this insight, we postulated that selectivity for BD1 could be obtained by exploiting the steric and electrostatic changes located around the aspartic acid residue of BD1 and the histidine of BD2 (the "Asp/His switch"), and set out to test this hypothesis.

We and others have identified multiple chemotypes which bind unselectively to BET bromodomains, the binding modes of many of which have been solved crystallographically.[3] Using this information, we evaluated all available templates to identify compounds appropriate for chemical modification to interact with the aspartic acid residues of the BD1 domains. Included in this analysis were quinoline isoxazoles and inspection of the complex of the key exemplar from this series, I-BET151 (**7**, Figure 3A),[23, 24] in BRD4 BD1[22] indicated the imidazoquinolinone 8-position as a suitable vector to target the BRD4 domain-specific residues Asp144 and His437. To

measure binding of I-BET151 and prospective derivatives to the BD1 and BD2 domains of BET, biochemical assay development was carried out.

Previous studies with a pan-BET inhibitor revealed apparent differences in affinity for isolated BRD2 bromodomains (BD1 and BD2) compared to the tandem BRD2 bromodomain constructs, which we attribute to differential stability of the single domain proteins.[21] Therefore, tandem bromodomain protein constructs with site-directed mutations impairing binding to the KAc pocket of either bromodomain were developed. This enabled compound IC_{50} determination for each domain upon displacement of a bromodomain non-selective fluorescent ligand (Supporting Information, Section S2). As controls for this approach, BET tandem constructs were produced with neither or both bromodomains mutated. BET proteins containing alanine mutants of the conserved tyrosine residue, whose sidechain hydroxyl anchors one of the key conserved water molecules essential for ligand recognition in the KAc pocket (for example, Tyr97 in BRD4 BD1), were expressed in *E. coli* and purified utilizing a 6-His tag at the N-terminus. Subsequently, a fluorescence polarization approach confirmed that the fluorescent ligand had similar binding affinities for each of the single bromodomain mutants as well as the non-mutated proteins but had a significant decrease in affinity for the double mutant constructs (Supporting Information, Figure S2iii). These results demonstrate that in the presence of one mutated bromodomain, the tandem protein is still able to bind the fluorescent ligand, through the non-mutated bromodomain, with a similar affinity to the wild type construct. However, if both bromodomains are mutated then the affinity for the fluorescent ligand is significantly diminished, demonstrating that neither of the bromodomains can bind in a similar manner to the non-mutated, wild type protein. Collectively, these results confirm the validity of this approach for measuring domain selectivity across the BET family and the assays were further developed into a TR-FRET format for use in this research.

In these assays, I-BET151 exhibited a modest inherent domain selectivity for BD1 over BD2 (13-fold for BRD4) (Table 1), which we attribute to a through-water interaction of the quinoline nitrogen to the side chain of Gln85 in BRD4 BD1, a residue replaced by Lys378 in BRD4 BD2 (Figure 3A). However, to ensure clear separation of binding from BD2 domains for assessment in phenotypic assays, a focused program of chemistry was carried out to increase the domain selectivity of I-BET151 towards a target of >100-fold.

Table 1. Activity and Selectivity Profile of I-BET151 in BET Mutant TR-FRET Assays

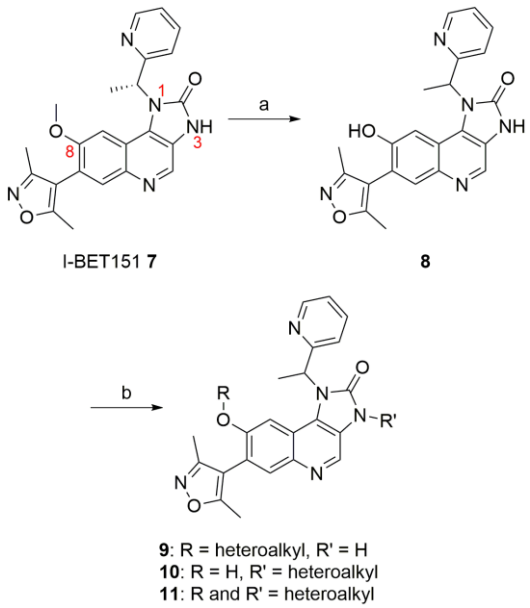
	pIC_{50}[a]		Selectivity (fold)[b]
	BD1	BD2	
BRD2	7.3	6.7	4.0
BRD3	7.5	7.1	2.5
BRD4	7.6	6.5	13
BRDT	6.9	5.8	13

[a]Expressed as the mean from at least five test occasions. [b]Calculated as $10^{(BD1\ pIC50 - BD2\ pIC50)}$.

An enantiomerically-enriched batch of I-BET151 (e.e. = 88% in favor of the *R*-isomer) available within our labs was used for SAR exploration at the imidazoquinolinone 8-position. Demethylation of the 8-OCH_3 group and reaction of quinolinol **8** with heteroalkylhalides typically resulted in a mixture of mono 8-O (**9**), mono 3-N (**10**) and bis 8-O, 3-N (**11**) alkylation products (Scheme 1). Rather than optimize the reaction conditions for selective mono *O*-alkylation, we pursued a synthetic strategy to cap the imidazoquinolinone 3-NH with a methyl group (Scheme 2). Examination of the crystal structure of I-BET151 bound to BRD4 BD1 showed that the 3-NH vector was directed towards solvent and a methyl substituent at this position was not expected to greatly affect activity or selectivity; this was subsequently confirmed upon screening the *N*-methylated compound **12** in the BRD4 mutant TR-FRET assays (Table 2) and thus compound **12** served as baseline for SAR comparison. This approach enabled selective demethylation of the 8-

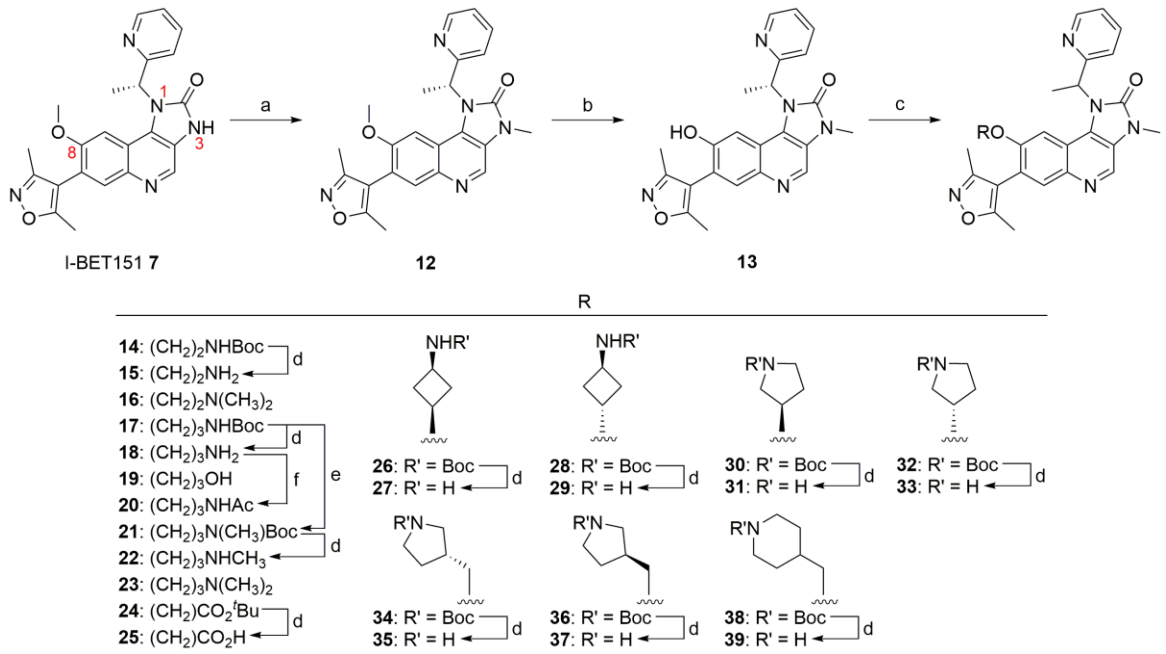
OCH_3 group using BBr_3 to give quinolol **13**, followed by *O*-alkylation to access target compounds **14**–**39** (Scheme 2). These target compounds included functionality that potentially interacted with the aspartic acid residues (or surrounding water molecules) of the BD1 domains and/or clashing with the His residues of the BD2 domains with the goal of increasing BD1 selectivity. Three analogues containing carboxylic acids (**25**, **42**, **44**) with the potential to selectively bind the histidine of BD2 were included in this initial investigation and those lacking a linking oxygen atom were accessed from the triflate **40** via palladium-catalyzed cross coupling and subsequent ester hydrolysis steps (Scheme 3).

Scheme 1. Alkylation of both the Imidazoquinolinone 3- and 8-Positions[a]



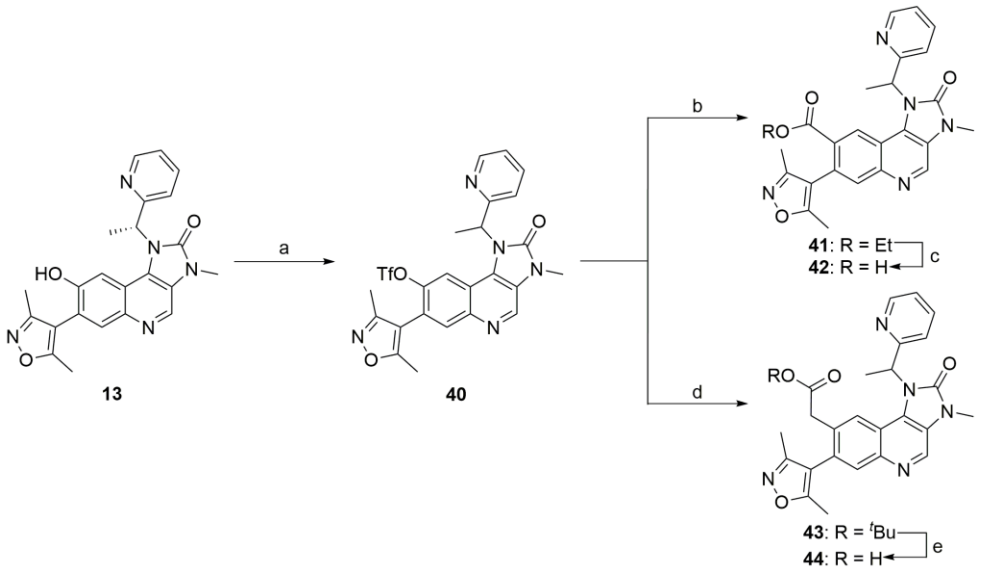
[a]Reagents and conditions: (a) BBr_3 (1 M in DCM), −78 ºC, 45 min, then rt, 16 h, then reflux, 22 h, 56%; (b) typical conditions: heteroalkylbromide, K_2CO_3, DMF, 100 °C.

Scheme 2. Synthesis of *O*-Substituted Analogues at the Imidazoquinolinone 8-Position[a]



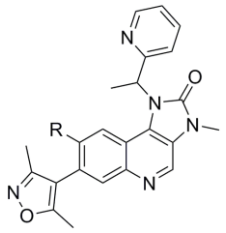
[a]Reagents and conditions: (a) NaH, CH_3I, NMP, rt, 5 h, 88%; (b) BBr_3, DCE, 90 °C, 3.5 h, 84%; (c) RX, K_2CO_3, DMF, 45–100 °C, 2–16 h, 24–86%; (d) 4 M HCl in 1,4-dioxane or TFA, DCM, rt, 1.5–16 h, 12–100%; (e) NaH, CH_3I, NMP, rt, 22 h, 22%; (f) Ac_2O, pyridine, DCM, rt, 2 h, 40%.

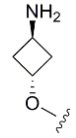
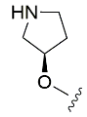
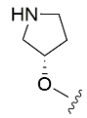
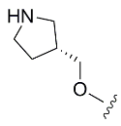
Scheme 3. Synthesis of Carboxylic Acids 42 and 44[a]

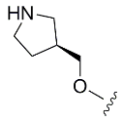
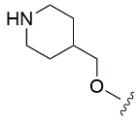


[a]Reagents and conditions: (a) Tf_2O, pyridine, DCM, 0 °C–rt, 4 h, 90%; (b) CO (g), $Pd(OAc)_2$ (7 mol%), 1,3-bis(diphenylphosphino)propane (7 mol%), NEt_3, EtOH, DMF, 70 °C, 3 h, 100%; (c) 2 M NaOH (aq), EtOH, rt, 15 h, then 50 °C, 3 h, 64%; (d) $BrZnCH_2CO_2{}^tBu$ (0.5 M in THF), $Pd(PPh_3)_4$ (10 mol%), NMP, microwave, 90 °C, 20 min, 17%; (e) TFA, DCM, rt, 36 h, 35%.

Table 2. Activity and Selectivity of I-BET151 Derivatives in BRD4 Mutant TR-FRET Assays



Compound	R	pIC_{50}[a]		Selectivity (fold)[b]
		BRD4 BD1	BRD4 BD2	
12	OCH_3	7.3	6.3	10
15	$O(CH_2)_2NH_2$	5.9	4.5	25
16	$O(CH_2)_2N(CH_3)_2$	5.7	<4.3	>25
18	$O(CH_2)_3NH_2$	6.2	4.8	25
19	$O(CH_2)_3OH$	6.3	5.2	13
20	$O(CH_2)_3NHAc$	6.1	5.5	4.0
22	$O(CH_2)_3NHCH_3$	6.0	4.6[c]	25
23	$O(CH_2)_3N(CH_3)_2$	6.0	<4.3	>50
25	OCH_2CO_2H	5.7	4.9	6.3
27		6.6	5.0	40
29		7.1	5.5	40
31		5.9	4.3[d]	40
33[e]		5.2	<4.3	>7.9
35[d]		6.1	4.5	40

37[d]		6.3	4.6	50
39		6.1	4.4[f]	50
42	CO_2H	4.9	<4.3	>4.0
44	CH_2CO_2H	<4.3	<4.3	-

[a]Expressed as the mean from at least two test occasions. [b]Calculated as $10^{(\text{BD1 pIC50} - \text{BD2 pIC50})}$. [c]A pIC_{50} value of <4.3 was determined on one test occasion out of five and was excluded from the reported mean value. [d]A pIC_{50} value of <4.3 was determined on two test occasions out of six and was excluded from the reported mean value. [e]Tested as a mixture of diastereoisomers. [f]A pIC_{50} value of <4.3 was determined on two test occasions out of four and was excluded from the reported mean value.

Upon screening these compounds in the BRD4 mutant TR-FRET assays, it was evident that most substituents led to a reduction in both BRD4 BD1 and BD2 activity relative to methoxy **12**. However, for basic substituents BD1 potency was less strongly affected than BD2, leading to increased BD1-selectivity compared to parent compound **12**. The ethylamine **15** displayed 25-fold greater activity at BRD4 BD1 over BRD4 BD2 while the selectivity for tertiary amine analogue **16** was >25-fold. The propylamine exhibited marginally greater BRD4 BD1 activity than ethylamine **15** but maintained the 25-fold level of selectivity. Replacing the amine of **18** with an alcohol (**19**), and acetylation to **14**, reduced selectivity. Substitution of the amine with a single methyl group (**22**) did not affect activity or selectivity but two methyls (**23**) did increase selectivity to >50-fold. Building on the encouraging initial results with basic groups connected by flexible alkyl chains, several rigidified analogues were then prepared (Scheme 2). Constraining the propylamine **18** into the *cis*- and *trans*-cyclobutylamines (**27** and **29**, respectively) increased domain selectivity to 40-fold, but a greater increase in BRD4 BD1 activity was observed for the *trans*-isomer. The same selectivity was observed for (*R*)-3-pyrrolidine **31** which displayed greater BRD4 BD1 activity than the *S*-isomer **33**. Homologation of these 3-pyrrolidines to the 3-methylene

pyrrolidines **35** and **37** revealed encouraging BD1-selectivity profiles but less discrimination between each stereoisomer was observed. Increasing the ring size to 4-methylene piperidine **39** also resulted in significant BD1-selectivity. Incorporation of carboxylic acid groups (**25**, **42** and **44**) led to diminution of activity at both domains.

Overall, these data demonstrated that *O*-linked basic substituents appended to the imidazoquinolinone 8-position resulted in enhanced selectivity over BRD4 BD2. However, incorporation of these substituents generally decreased BRD4 BD1 potency with respect to **12** and did not meet our desired activity (pIC_{50} ~7) for comparison to pan-BET inhibitors in phenotypic assays. Therefore, we examined other quinoline isoxazoles available within our collection in the mutant TR-FRET assays to identify a more potent analogue of I-BET151 suitable for derivatization with an appropriate basic substituent – the (*S*)-3-methylene pyrrolidine contained in **37** was selected as a preferred group for this exercise.

We previously described a strategy to cyclize 4-amino-3-carboxamide quinoline isoxazoles to imidazoquinolinone isoxazoles (Figure 4) which conferred several benefits including increased potency.[23] In a similar approach previously unpublished by our labs, an imidazoquinoline scaffold was established (Figure 4) which typically conferred even higher activity and is exemplified by 8-methoxy imidazoquinoline **45** (Table 3, see Supporting Information Section S3ii for synthesis and characterization).

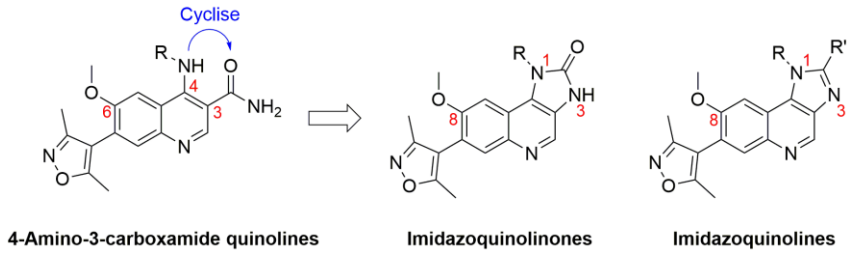
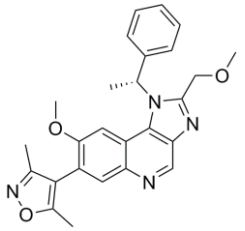


Figure 4. Cyclisation approach used by Mirguet et al. in the genesis of imidazoquinolinones[23] and the related imidazoquinoline scaffold.

Table 3. Activity and Selectivity Profile of Imidazoquinoline 45 in BET Mutant TR-FRET Assays

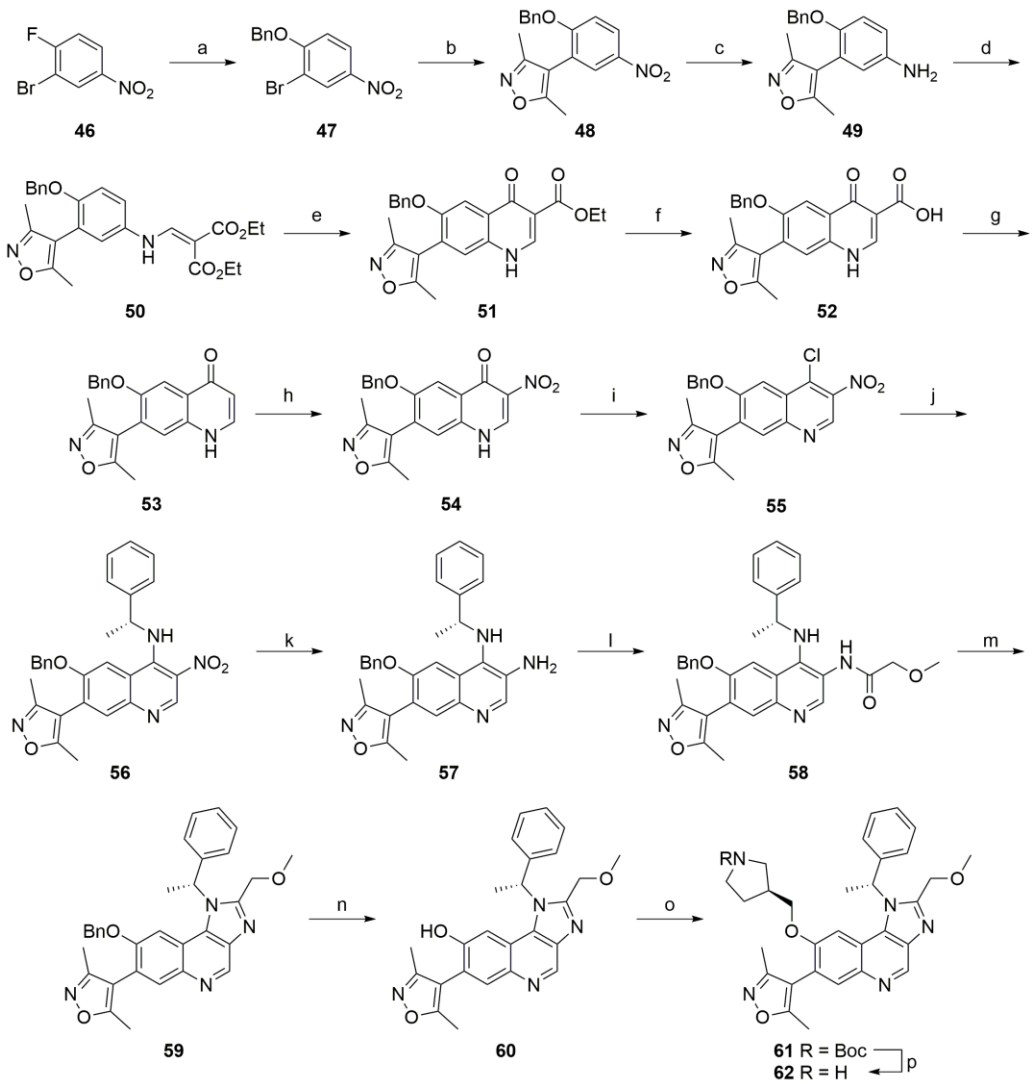


	pIC_{50}[a]		Selectivity (fold)[b]
	BD1	BD2	
BRD2	7.9	7.4	3.2
BRD3	8.0	7.8	1.6
BRD4	8.0	7.4	4.0
BRDT	7.6	6.5	13

[a]Expressed as the mean from at least five test occasions. [b]Calculated as $10^{(\text{BD1 pIC50} - \text{BD2 pIC50})}$.

The BD1 potency of imidazoquinoline **45** approached the tight binding limit of the BRD2, 3 and 4 TR-FRET assays and the selectivity over BET BD2 domains was therefore likely to be underestimated. However, the BD1 selectivity at BRDT (13-fold) was the same as that of I-BET151 and selectivities at the other BET isoforms were also expected to be similar to I-BET151. Importantly, 8-methoxy imidazoquinoline **45** exhibited sufficiently high activity at BET BD1 domains so that appendage of the preferred basic substituent at the 8-position was expected to deliver our potency and selectivity requirements. In this regard, imidazoquinoline **62** was targeted for synthesis and was achieved using the route shown in Scheme 4.

Scheme 4. Synthesis of Imidazoquinoline 62[a]



[a]Reagents and conditions: (a) NaH, BnOH, DMA, 0 °C, 1 h, 73%; (b) (3,5-dimethylisoxazol-4-yl)boronic acid, PEPPSI-IPr (2.5 mol%), Cs_2CO_3, DME, H_2O, 90 °C, 5 h, 86%; (c) Fe, NH_4Cl, EtOH, H_2O, rt, 15 h, 98%; (d) diethyl 2-(ethoxymethylene)malonate, 130 °C, 1 h, 74%; (e) Ph_2O, 260 °C, 20 min, 90%; (f) 2 M NaOH (aq), EtOH, 95 °C, 3 h, 99%; (g) Ph_2O, 260 °C, 20 min, 89%; (h) HNO_3, $EtCO_2H$, 100 °C, 1.5 h, 57%; (i) $POCl_3$, 100 °C, 1 h, 96%; (j) (*R*)-α-methylbenzylamine, $^iPr_2NEt$, NMP, rt, 2.5 h, 99%; (k) Fe, AcOH, rt, 1 h, 83%; (l) 2-methoxyacetyl chloride, pyridine, DCM, rt, 3.5 h, 98%; (m) $EtCO_2H$, 140 °C, 2 h, 90%; (n) 5% Pd/C, H_2, EtOH, rt, 35 h, 62%; (o) (*S*)-*tert*-butyl 3-{[(methylsulfonyl)oxy]methyl}pyrrolidine-1-carboxylate, K_2CO_3, DMF, 100 °C, 3 h, 57%; (p) 4 M HCl in 1,4-dioxane, 1,4-dioxane, rt, 1 h, 96%.

Firstly, the protected phenol moiety was introduced by nucleophilic substitution of 2-bromo-1-fluoro-4-nitrobenzene **46** with benzyl alcohol to furnish the benzylether **47**. A Suzuki-Miyaura reaction of this material with (3,5-dimethylisoxazol-4-yl)boronic acid formed the cross-coupling

product **48** and was followed by nitro reduction to give the aniline **49**. Conjugate substitution with diethyl-2-(ethoxymethylene)malonate followed by cyclisation in refluxing diphenylether gave 3-ethyl[quinolin-4(1*H*)-one]carboxylate **51**. Saponification and subsequent decarboxylation gave the quinolin-4(1*H*)-one **53**, which was then nitrated at the 3-position. After chlorination, a nucleophilic substitution reaction installed the (*R*)-α-methylbenzylamine 4-substituent and reduction of the 3-position nitro unit afforded amine **57**. Acylation with 2-methoxyacetyl chloride, followed by cyclisation in hot propionic acid, constructed the 1*H*-imidazo[4,5-*c*]quinoline ring system in an efficient manner and, upon hydrogenation over Pd/C, imidazoquinolinol **60** was obtained. Final compound synthesis was accomplished through *O*-alkylation with (*S*)-*tert*-butyl 3-{[(methylsulfonyl)oxy]methyl}pyrrolidine-1-carboxylate, followed by *N*Boc deprotection giving target compound **62**. Chiral HPLC analysis of this material (Supporting Information, Figure S3iv) confirmed that the stereochemical integrity of the α-methylbenzyl group remained intact throughout the preparative sequence.

Upon testing, greater BRD4 BD1 activity was found for imidazoquinoline **62** (pIC_{50} = 7.4) compared to imidazoquinolinone **37**, validating the switch to an alternative quinoline isoxazole scaffold (Figure 5 and Table 4). We were also pleased to find greater domain selectivity in BRD4 (>150-fold) was achieved with this modification and highlighted the sensitivity of SAR towards minor scaffold changes. The strong preference for BD1-binding was replicated in the rest of the BET family albeit at reduced levels compared to BRD4 (Table 4). With activities at BD1 domains comparable to established pan-BET inhibitors, and accompanied by suitably large selectivities over BD2 domains, **62** was investigated in further experiments.

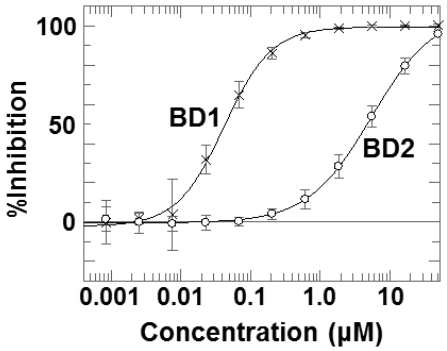


Figure 5. Concentration-response curves of **62** in BRD4 mutant TR-FRET assays highlighting greater preference for BD1 binding (crosses) over BD2 (circles). Data points represent the mean from 16 test occasions.

Table 4. Activity and Selectivity Profiles of Imidazoquinoline 62 in BET Mutant TR-FRET Assays

	pIC_{50}[a]		Selectivity (fold)[b]
	BD1	BD2	
BRD2	7.1	5.5	40
BRD3	7.4	6.0	25
BRD4	7.4	5.2	158
BRDT	6.8	4.8	100

[a]Expressed as the mean from at least eight test occasions. [b]Calculated as $10^{(\text{BD1 pIC50} - \text{BD2 pIC50})}$.

We next sought to rationalize the selectivity of **62** using X-ray crystallography and a high-resolution structure was obtained in BRD4 BD1 (Figure 6A). The 3,5-dimethylisoxazole moiety forms characteristic hydrogen bonding interactions to the conserved Asn140 and the water network at the base of the binding site, while the aryl ring of the α-methylbenzyl substituent occupies the WPF shelf and the quinoline nitrogen retains the through-water interaction to Gln85. These features are similar to I-BET151 and other previously reported compounds from this series.[22-24] As intended, the 3-methylene pyrrolidine substituent projects towards Asp144. Rather than making

a direct salt-bridge with Asp144, the pyrrolidine amine interacts with Asp144 via a water molecule that also hydrogen-bonds to the sidechain oxygen of Asn140 (Figure 6A and B). Unexpectedly, a second aspartic acid, Asp145, rotates towards the inhibitor, and its acid group forms a second interaction connected to the pyrrolidine nitrogen by another two bridging water molecules. This second H-bonding chain has less ideal geometry and higher B-factors than the first (Supporting Information, Figure S5). The Asp145 movement displaces the C-helix capping water hydrogen-bonded to Ile146 NH (Wc, Figure 6A), replacing this interaction with one of the Asp145 sidechain carboxylate oxygens.

We assume that the water-mediated pyrrolidine interactions of imidazoquinoline **62** would be replicated by imidazoquinolinone **37**, which is tenfold less potent against BRD4 BD1 than **12** (Table 2). Given all the polar interactions this may seem unexpected, but the pyrrolidine group is probably well solvated when unbound, and the reduced BD1 potency suggests that the interactions it forms in the BD1 complex only partly compensate for unfavorable effects of extending the methoxy substituent. We hypothesize that the water seen in this region of the complex with methoxy substituted compound **7** is quite stable and resists deformation. However, the increased selectivity of imidazoquinoline **62** arises not because its pyrrolidine makes favorable interactions with BD1, but because its interactions with BD2 are more unfavorable. To gain further insights into this, a crystal structure of imidazoquinoline **62** in BRD2 BD2 was obtained (Figure 6C and D). The overall binding mode is similar to that in BRD4 BD1 (Figure 6C) except for the pyrrolidine and its surroundings. The dual substitution of BRD4 BD1 Asp144 and Lys141 for BRD2 BD2 His433 and Pro430 creates a BD2 site which cannot accommodate the water network that coordinates the pyrrolidine nitrogen in the BD1 structure. In BD2, His433 is seen in both chi1 rotamers, a minor *gauche*(+) and a major trans (pointing “out” and “in” with respect to the KAc

site, Figure 6D). In its major "in" conformation the sidechain of His433 overlaps with the volume occupied by BRD4 BD1 Asp145 and its associated water chain. As a result, the analogous BRD4 BD2 residue Asp434 cannot rotate inwards towards the pyrrolidine. Even in its minor "out" conformation, steric constraints from the sidechain of Pro430 push His433 further into the space containing pyrrolidine-binding water molecules in BD1. Consequently, in BD2 the pyrrolidine nitrogen of imidazoquinoline **62** is forced 1.8 Å away from its BD1 position and cannot hydrogen-bond via water to any negatively ionized sidechains. The selectivity of imidazoquinoline **62** can therefore be rationalized by the "Asp-His switch", even though the pyrrolidine does not directly interact with these sidechains.

The role of the "Asp/His switch" in domain selectivity was discussed by Divakaran et al. in the context of their dual kinase/BD1 bromodomain inhibitor **5**.[10] They observed no binding to a BRD4 BD2 His437-Asp mutant suggesting that selectivity for BRD4 BD1 over BRD4 BD2 was not wholly due to the "Asp/His switch". An alternative suggestion from these researchers was the presence of proline at BRD4 BD2 position 434 also influences selectivity by restricting protein flexibility. While inhibitor **5** is structurally different to imidazoquinoline **62**, and binds deeper in the KAc site displacing conserved water, the selectivity of the two molecules may arise from similar mechanisms.

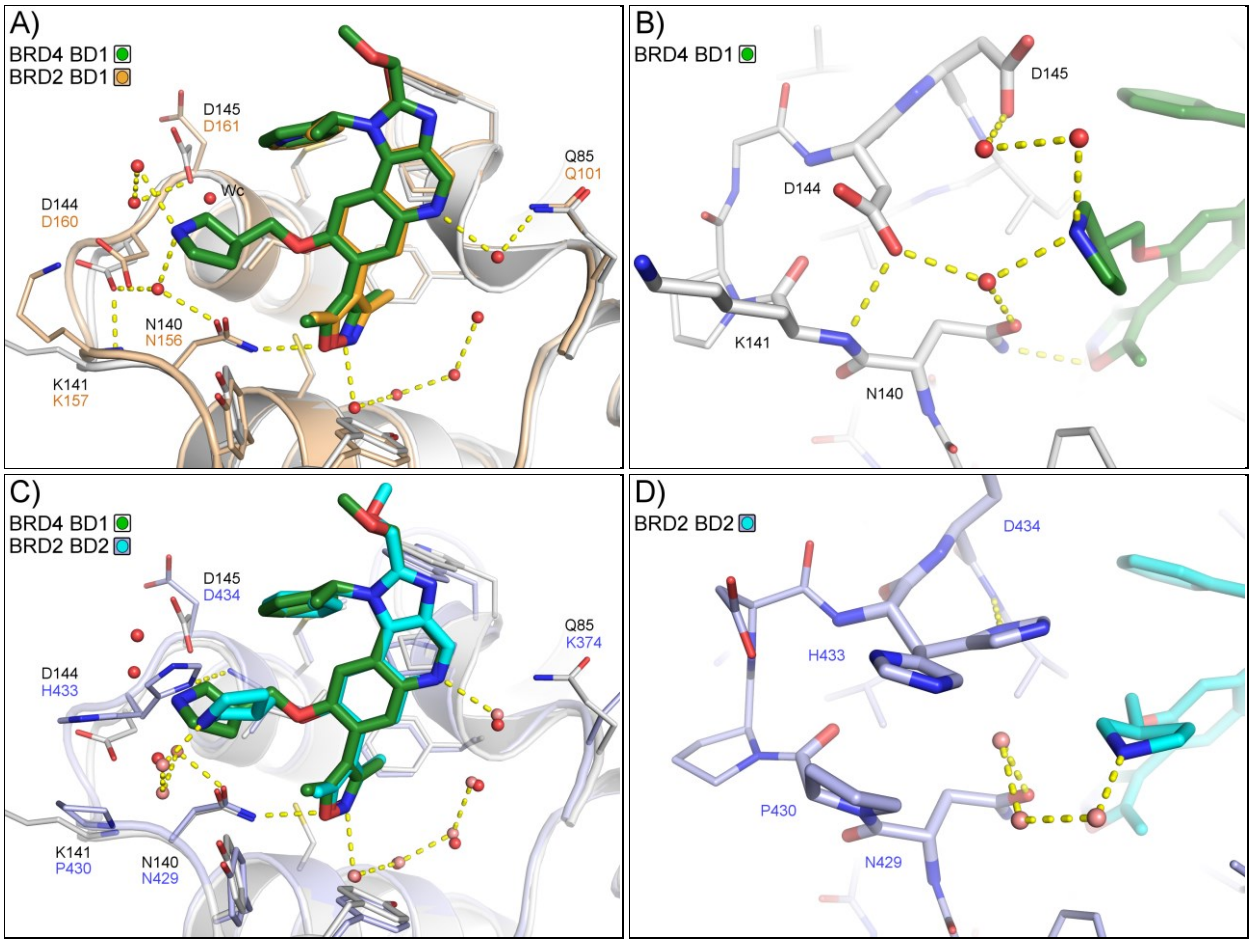


Figure 6. (A) Crystal structure of **62** in BRD4 BD1 (ligand carbon = green, protein carbon = white, PDB 6swn[25]) overlaid on I-BET151 in BRD2 BD1 (ligand and protein carbon = orange, PDB 4alg[24]). (B) Magnified view of pyrrolidine water-bridged interactions to BRD4 BD1 Asp144, Asp145 and Asn140. (C) Crystal structure of **62** in BRD2 BD2 (ligand carbon = cyan, protein carbon = blue, PDB 6swo[25]) superimposed on the structure of **62** in BRD4 BD1 (ligand carbon = green, protein carbon = white). (D) Magnified view of pyrrolidine water-bridged interactions with BRD2 BD2.

Further confirmation of the domain selectivity of imidazoquinoline **62** was obtained by SPR (Figure 7 and Supporting Information Figure S6ii). Titrations of **62** over immobilized BRD4 BD1 and BRD4 BD2 resulted in pK_Ds of 8.0 and 5.9, respectively, and domain selectivity >100-fold.

Additional profiling was conducted using a qPCR-based assay at DiscoveRx Corp. (Supporting Information, Table S7i). Here, imidazoquinoline **62** displayed selective binding within the BET family and over 100-fold selectivity for BRD4 BD1 over BRD4 BD2. These data also confirmed the specificity of **62** for the BET family with negligible binding detected at 24 other bromodomains.

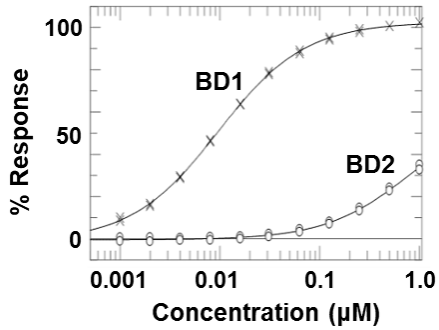


Figure 7. SPR concentration-response curves for **62** highlighting greater preference for BRD4 BD1 (crosses) over BRD4 BD2 (circles). Compound was tested in duplicate and the individual curves overlay closely.

Having established a clear preference for binding at the BD1 domains of BET in multiple assay formats using recombinant proteins, **62** was assessed for its ability to act as a chemical probe in cells. HEK293 cells expressing a nanoluciferase-BRD4 fusion protein containing unmutated bromodomains were incubated with **62** and inhibition of a bromosporine tracer was determined with a pIC_{50} value of 7.3. These data confirmed the ability of **62** to engage the bromodomain modules of BRD4 in cells and that this compound is therefore suitable for use as a probe molecule for assessment in phenotypic assays.

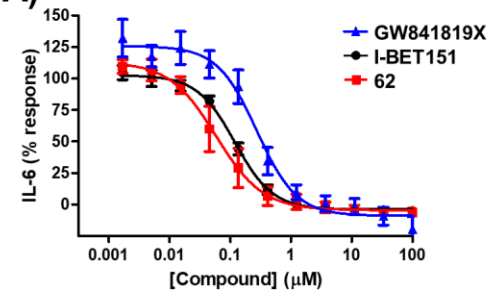
The anti-inflammatory properties of BET inhibitors were first discovered upon studies with I-BET762 in mouse macrophages where displacement of BRD2, 3 and 4 from gene promoters suppressed LPS-induced pro-inflammatory cytokine and chemokine gene expression.[26] With anti-

inflammatory effects becoming a hallmark of BET inhibition,[7, 24, 27-37] we examined the ability of **62** to block pro-inflammatory cytokine production alongside the pan-BET inhibitor GW841819X[21] (see Supporting Information Table S4v confirming unselective binding in mutant TR-FRET assays) and the BD1-biased inhibitor I-BET151 (Figure 8A–D). In both LPS-stimulated human peripheral blood mononuclear cells (PBMCs) and human whole blood, **62** suppressed IL-6 and monocyte chemotactic protein-1 (MCP-1) production with comparable activities to GW841819X and I-BET151 in both cellular environments.

The profound anti-leukemic effects of I-BET151 in mixed lineage leukemic (MLL) fusion cell lines have, in part, been attributed to down-regulation of MLL target genes through displacement of BRD3/4 and members of the super elongation complex (SEC) and polymerase-associated factor complex (PAFc) from chromatin.[22] Further reports demonstrating the efficacy of BET inhibitors in acute myeloid leukemia (AML)[38-43] prompted us to compare the anti-proliferative effects of **62**, alongside GW841819X and I-BET151, in MV-4-11 cells, an AML cell line harboring a MLL-AF4 rearrangement. Upon treatment of these cells over a three-day period, **62** was found to potently suppress cell growth with a pIC_{50} value of 7.0 (Figure 8E). This value was in close accordance with those obtained for GW841819X and I-BET151 (pIC_{50} = 6.7 and 7.0, respectively), suggesting that efficacy in MV-4-11 cells is mediated mainly through the BD1 domains.

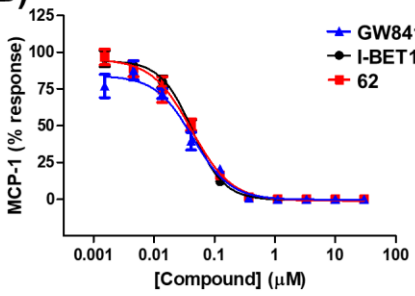
Overall, these results showed that the anti-inflammatory and anti-proliferative phenotype characteristic of pan-BET inhibition was retained upon selective inhibition of BD1 domains.

A)



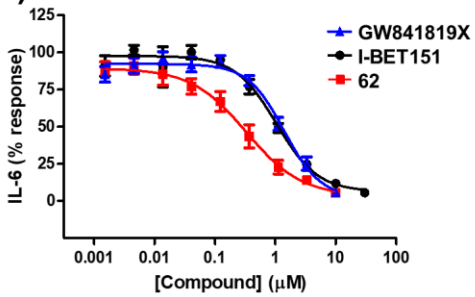
PBMC IL-6			
Compound	Mean pIC_{50}	SD	n
GW841819X	6.5	0.2	4
I-BET151	6.9	0.0	10
62	7.2	0.4	4

B)



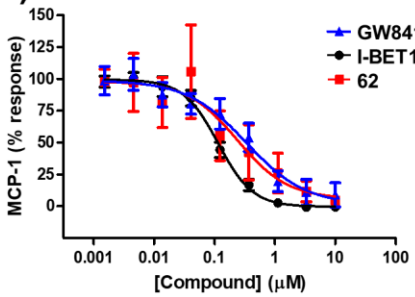
PBMC MCP-1			
Compound	Mean pIC_{50}	SD	n
GW841819X	7.3	0.1	6
I-BET151	7.4	0.2	12
62	7.4	0.2	8

C)



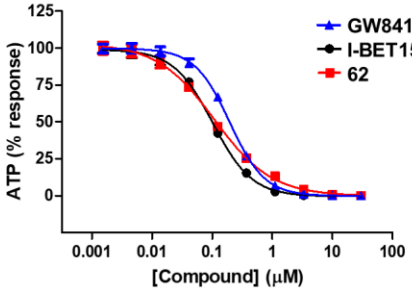
hWB IL-6			
Compound	Mean pIC_{50}	SD	n
GW841819X	5.8	0.2	5
I-BET151	6.0	0.1	8
62	6.6	0.6	11

D)



hWB MCP-1			
Compound	Mean pIC_{50}	SD	n
GW841819X	6.7	0.2	3
I-BET151	6.7	0.1	8
62	6.8	0.6	5

E)



MV-4-11			
Compound	Mean pIC_{50}	SD	n
GW841819X	6.7	0.0	3
I-BET151	7.0	0.1	3
62	7.0	0.1	3

Figure 8. Inhibitory effect of GW841819X, I-BET151 and **62** on IL-6 and MCP-1 production in LPS-stimulated human PBMCs (A and B) and whole blood (C and D). (E) Growth inhibition of GW841819X, I-BET151 and **62** in MV-4-11 cells.

The potential for **62** to be used as a probe molecule in vivo was explored next. Following intra-peritoneal (ip) administration to the male CD1 mouse at 10 mg/kg, compound **62** was well tolerated and demonstrated good exposure (Table 5 and Figure 9A). Visual inspection of the concentration-time profile (Figure 9A) indicated biphasic elimination with a moderate (predominant) elimination half-life of approximately 1 h and a longer terminal phase (Table 5). As only unbound drug is available for efficacy the unbound fraction in mouse blood was determined by rapid equilibrium dialysis and found to be 0.08. To provide a suitability assessment for exploration of in vivo activity the in vivo response (termed target engagement) in blood over a time course was estimated. Using a sigmoid Emax model the blood concentration-time profile obtained following ip administration was integrated with measures of in vitro potency (from either BRD4 BD1 and BD2 mutant TR-FRET assays or human whole blood IL-6 inhibition assay corrected for free fraction and assuming equivalent potency across species). The plot shown in Figure 9B illustrates that an ip dose of 10 mg/kg is estimated to provide over 85% BD1 target engagement at Cmax and maintain over 45% for approximately 2 h post administration, whilst maintaining BD2 levels no greater than 4%, confirming the properties of **62** allow the selective probing of BD1. Alternative estimations of in vivo target engagement using a marker of whole blood activity confirm that an ip dose of 10 mg/kg offers potential for in vivo efficacy (Figure 9C). Studies with **62** have demonstrated increased survival in a mouse MLL-AF9 AML model compared to a BD2-selective inhibitor.[25] Taken together these data illustrate that **62** has suitable disposition, potency, and selectivity properties appropriate to explore BD1 pharmacology in vivo.

Table 5. The Pharmacokinetic Parameters of 62 Following ip Administration (10 mg/kg) to the Male CD1 Mouse

Parameter	Mean (n = 3) ± SD
C_{max} (μM)	2.40 ± 0.46
T_{max} (h)	0.25–0.50
Predominant (0–7 h) $T_{1/2}$ (h)	0.9 ± 0.1
Terminal (7–24 h) $T_{1/2}$ (h)	8.2 ± 0.7
AUC_{∞} (μM*h)	3.34 ± 0.31

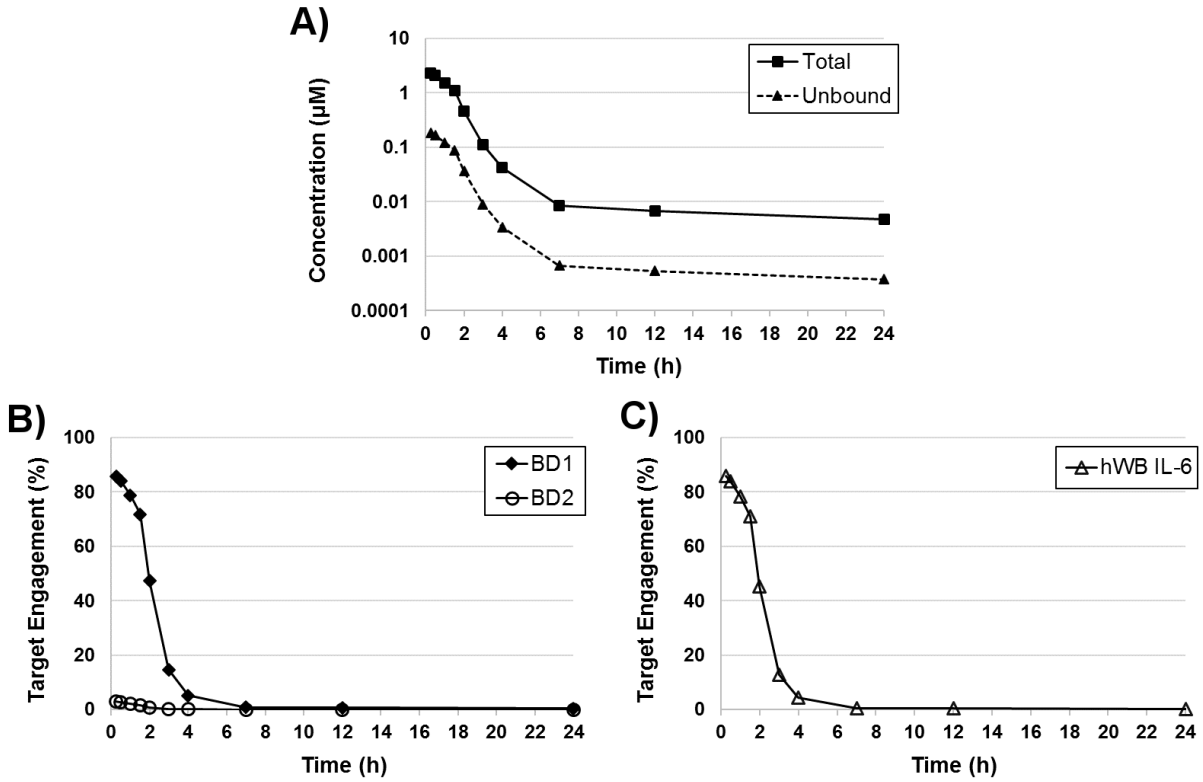


Figure 9. (A) Blood concentration-time profile of compound **62** following ip administration (10 mg/kg) to the male CD1 mouse. Total concentrations are depicted with closed squares and unbound concentrations with closed triangles. Data are mean n = 3. (B and C) Predicted in vivo target engagement-time profiles for compound **62** integrating the mouse ip PK data and the in vitro

IC_{50} values determined in (B) BRD4 BD1 (closed diamonds) and BRD4 BD2 (open circles) mutant TR-FRET assays, and (C) LPS-stimulated IL-6 inhibition in human whole blood (open triangles).

CONCLUSIONS In this report, we have described the development of a small molecule which retains the wider bromodomain selectivity of I-BET151, but also exhibits significant selectivity for BD1 over BD2 domains within the BET family of tandem bromodomain-containing proteins. Structural knowledge was used to identify aspartic acid residues present only in BD1 domains for selective targeting with chemical functionality appended to the 8-position of I-BET151. Pendant rigidified amines enhanced selectivity for BD1 domains but also decreased activity. Therefore, a strategy to append the favored 3-methylene pyrrolidine to a more potent analogue of I-BET151 was executed and resulted in imidazoquinoline **62** featuring up to 150-fold BD1-selectivity, as well as BD1-activity comparable to the biased inhibitor I-BET151 and the pan-inhibitor GW841819X. An X-ray crystal structure of **62** in BRD4 BD1 revealed through-water interactions between the pendant amine and the BD1-conserved residues Asp144 and Asp145. In the BD2 domains, the substitutions of histidine for Asp144 and proline for Lys141 prevent **62** from forming these interactions and are the likely origin of the observed domain selectivity. Studies with **62** in human PBMCs and whole blood, and in MV-4-11 cells, demonstrated that selective inhibition of BD1 retains the anti-inflammatory and anti-proliferative phenotype characteristic of pan-BET inhibition. The pharmacokinetic profile of **62** make this compound suitable for use to probe BD1 selective pharmacology in vivo. Biological investigations of **62** (also referred to as GSK778/iBET-BD1) alongside BD2-selective molecules (GSK046/iBET-BD2 and GSK620)[29,30] have very recently been reported,[25] and further contribute to other recent progress in this area.[11]

EXPERIMENTAL SECTION

General Experimental.

All commercial chemicals and solvents used were reagent grade and used without further purification. SCX-2 and aminopropyl cartridges by ISOLUTE were used for catch and release, and scavenging SPE protocols. Column chromatography was carried out on either a Biotage SP4 automated flash chromatography system using SNAP silica cartridges, or a Combiflash Companion automated flash chromatography system using Redisep silica cartridges. Mass-directed autopreparative HPLC (MDAP) purification was conducted on a Waters HPLC using either Method A, Sunfire C18 (150 mm × 30 mm, 5 μm packing diameter) eluting with an appropriate gradient of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in CH_3CN (mobile phase B) at ambient temperature and a flow rate of 40 mL/min; or Method B, Xbridge C18 column (150 mm × 30 mm, 5 μm packing diameter) eluting with an appropriate gradient of 10 mM ammonium bicarbonate in water adjusted to pH 10 with ammonia solution (mobile phase A) and CH_3CN (mobile phase B) at ambient temperature and a flow rate of 40 mL/min; or Method C, Sunfire C18 (150 mm × 30 mm, 5 μm packing diameter) eluting with an appropriate gradient of 0.1% TFA in water (mobile phase A) and 0.1% TFA in CH_3CN (mobile phase B) at ambient temperature and a flow rate of 40 mL/min. The UV detection was an averaged signal from wavelength of 210 nm to 350 nm, and mass spectra recorded on a Waters micromass ZQ mass spectrometer operating in alternate-scan positive and negative electrospray ionization modes for Methods A and B, and in positive electrospray ionization mode for Method C. Melting points were measured using a Stuart SMP40 automatic melting point apparatus. IR spectra were obtained on a Perkin Elmer Spectrum 1 machine. Optical rotations were measured using a Jasco P-1030 polarimeter. ^{1}H NMR spectra were recorded on a Bruker AV-400 spectrometer at 400 MHz or Bruker AV-600 spectrometer at 600 MHz. ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer at 100 MHz or a Bruker AV-600 spectrometer at 150 MHz. NMR spectra were

acquired at 293 K unless otherwise stated. Signal multiplicities were assigned using the following abbreviations: s (singlet), br s (broad singlet), t (triplet), q (quartet), quin (quintet), m (multiplet), dd (doublet of doublets), dt (doublet of triplets), dq (doublet of quartets), td (triplet of doublets) and tt (triplet of triplets). The purity of all biologically tested compounds was ≥95% as determined by LC-MS UV traces except for compound **25** (94% purity). The specific LC-MS UV method used for purity determination is noted for each compound and was conducted on a Waters Acquity UPLC equipped with a BEH C18 column (50 mm × 2.1 mm, 1.7 μm packing diameter) using a gradient elution carried out at a flow rate of 1 mL/min at 40 °C using either Method A or B. Method A: 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in CH_3CN (mobile phase B) 0.0–1.5 min 3–100% mobile phase B, 1.5–1.9 min 100% mobile phase B, 1.9–2.0 min 100–3% mobile phase B. Method B: 10 mM ammonium bicarbonate in water adjusted to pH 10 with ammonia solution (mobile phase A) and CH_3CN (mobile phase B) 0.0–1.5 min 1–97% mobile phase B, 1.5–1.9 min 97% mobile phase B, 1.9–2.0 min 97–1% mobile phase B. The UV detection was a summed signal from wavelength of 210 nm to 350 nm, and mass spectra recorded on a Waters micromass ZQ mass spectrometer operating in alternate-scan positive and negative electrospray ionization modes. High resolution mass spectra were acquired on a Micromass Q-Tof Ultima hybrid quadrupole TOF mass spectrometer.

Chemistry Methods and Characterization for Compounds 8–62.

7-(3,5-Dimethyl-4-isoxazolyl)-8-hydroxy-1-[(1*R*)-1-(2-pyridinyl)ethyl]-1,3-dihydro-2*H*-imidazo[4,5-*c*]quinolin-2-one (8). 7-(3,5-Dimethyl-4-isoxazolyl)-8-(methyloxy)-1-[(1*R*)-1-(2-pyridinyl)ethyl]-1,3-dihydro-2*H*-imidazo[4,5-*c*]quinolin-2-one (I-BET151, 1 g, 2.41 mmol, e.e. unknown) in DCM (25 mL) under nitrogen at −78 ºC was treated with 1 M BBr_3 in DCM (12 mL, 12.00 mmol) added over 30 min. The reaction was stirred at −78 ºC for 45 min then allowed to

warm to rt and stirred for 16 h. The reaction was heated to reflux for 4 h then additional 1 M BBr_3 in DCM (6 mL, 6 mmol) was added. After a further 2 h, an additional portion of 1 M BBr_3 in DCM (6 mL, 6 mmol) was added and reflux continued for 16 h. The reaction was cooled in an ice-bath and EtOH (2.5 mL) in DCM (12.5 mL) was added slowly. After stirring for 20 min the reaction mixture was concentrated to give a brown solid. The crude material was purified on a 100 g silica cartridge eluting with 1–10% 2 M ammonia/MeOH in DCM. The appropriate fractions were concentrated in vacuo to give the title compound as a cream solid (0.57 g, 1.35 mmol, 56% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.53 (br s, 1 H), 10.03 (br s, 1 H), 8.54 (s, 1 H), 7.79–7.74 (m, 2 H), 7.38 (d, J = 8.1 Hz, 1 H), 7.30 (m, 1 H), 7.18 (br s, 1 H), 6.18 (q, J = 7.2 Hz, 1 H), 2.29 (s, 3 H), 2.12 (s, 3 H), 2.08 (d, J = 7.2 Hz, 3 H); % e.e. undetermined; LC-MS (Method A) $[M + H]^+$ = 402, R_t 0.57 min.

7-(3,5-Dimethylisoxazol-4-yl)-8-methoxy-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one (12). A solution of 7-(3,5-dimethylisoxazol-4-yl)-8-methoxy-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one (I-BET151 **7**, 4.0 g, 9.63 mmol, e.e. = 88%) in anhydrous NMP (30 mL) under nitrogen at rt, was treated with NaH (60% dispersion in mineral oil) (0.50 g, 12.5 mmol) and the mixture stirred for 20 min. Methyl iodide (0.66 mL, 10.6 mmol) was added and the mixture stirred for 5 h. The reaction mixture was diluted with brine (50 mL) and extracted with EtOAc (50 mL). The organic layer was washed with water (4 × 50 mL) then passed through a hydrophobic frit. The solvent was evaporated in vacuo to give the title compound as a cream foam (3.63 g, 8.45 mmol, 88% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 353 K) δ 8.82 (s, 1 H), 8.61 (d, J = 4.8 Hz, 1 H), 7.81 (s, 1 H), 7.80–7.74 (m, 1 H), 7.44 (d, J = 8.1 Hz, 1 H), 7.33 (dd, J = 7.3, 4.8 Hz, 1 H), 6.94 (s, 1 H), 6.33 (q, J = 7.2 Hz, 1 H), 3.62 (s, 3 H), 3.55 (s, 3 H), 2.26 (s, 3 H), 2.07 (d, J = 7.2 Hz, 3 H), 2.06 (s, 3 H); 76% e.e. determined by HPLC

analysis on a Chiralpak AD column (250 × 4.6 mm, 10 μm), elution with 40% EtOH in heptane, flow rate 1 mL/min, UV detection at 215 nm; LC-MS (Method A) $[M + H]^+ = 430$, R_t 0.62 min.

7-(3,5-Dimethylisoxazol-4-yl)-8-hydroxy-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one (13). To 7-(3,5-dimethylisoxazol-4-yl)-8-methoxy-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one **12** (3.63 g, 8.45 mmol) in DCE (75 mL) was added BBr_3 (8.0 mL, 85 mmol) dropwise. A thick precipitate formed and after addition was complete, the reaction was stirred at 90 ºC under nitrogen for 3.5 h. The reaction was cooled in an ice-bath and carefully quenched with 10% EtOH in DCM (75 mL) and then MeOH (75 mL). After stirring for 20 min, the solvent was removed in vacuo. The solid-foam mixture was suspended in EtOAc (200 mL) and sonicated for 10 min. To the mixture was added sat. Na_2CO_3 (aq) (200 mL) and the biphasic mixture stirred vigorously for 10 min. The organic layer was separated and left to stand for 5 min during which time a precipitate formed. The solid was isolated by vacuum filtration and the filtrate dried ($MgSO_4$). The solvent was evaporated in vacuo and the solid triturated with ether (50 mL). This solid was combined with the previous solid precipitate to give the title compound as an off-white solid (2.96 g, 7.12 mmol, 84% yield). mp 287 ºC (decomp.); $[\alpha]_D^{21}$ −25 (*c* 0.5, $CHCl_3$/MeOH [4:1]); ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.70 (s, 1 H), 8.54 (d, J = 4.8 Hz, 1 H), 7.79 (s, 1 H), 7.76–7.70 (m, 1 H), 7.40 (d, J = 7.8 Hz, 1 H), 7.37 (s, 1 H), 7.26 (dd, J = 7.4, 4.8 Hz, 1H), 6.27 (q, J = 7.2 Hz, 1 H), 3.54 (s, 3 H), 2.31 (s, 3 H), 2.14 (s, 3 H), 2.12 (d, J = 7.2 Hz, 3 H) O*H* signal not resolved; ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.8, 159.1, 158.7, 153.3 (2 C), 149.0, 139.8, 137.0, 132.9, 130.9, 126.9, 123.3, 122.4, 120.7, 120.2, 115.9, 112.6, 103.6, 53.8, 27.4, 17.5, 11.4, 10.4; 76% e.e. determined by HPLC analysis on a Chiralpak ID column (250 × 4.6 mm, 5 μm), elution with 50% EtOH in heptane, flow rate 1

mL/min, UV detection at 215 nm; LC-MS (Method A) $[M + H]^+ = 416$, R_t 0.62 min; HRMS $[M + H]^+$ calcd for $C_{23}H_{22}N_5O_3$ 416.1723, found 416.1711.

***tert*-Butyl [2-({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)ethyl]carbamate (14).** 7-(3,5-Dimethylisoxazol-4-yl)-8-hydroxy-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one **13** (100 mg, 0.241 mmol), potassium carbonate (43 mg, 0.310 mmol) and *tert*-butyl (2-bromoethyl)carbamate (54 mg, 0.241 mmol) were combined in DMF (5 mL) and heated at 100 ºC overnight. The reaction mixture was cooled to rt and concentrated in vacuo. The crude compound was purified on a 25 g silica cartridge eluting in with 1% MeOH in DCM for 2 column volumes, 1–5% MeOH in DCM for 10 column volumes, then 5% MeOH in DCM for 5 column volumes. The appropriate fractions were concentrated in vacuo to give an oil which was then purified by MDAP (Method A). The appropriate fractions were concentrated in vacuo. The residue was dissolved in MeOH and applied to a 2 g SCX-2 cartridge which was then washed with MeOH (10 mL) followed by 2 M ammonia in MeOH solution (10 mL). The basic wash was concentrated to give the title compound as an orange oil (59 mg, 0.095 mmol, 40% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (d, J = 4.5 Hz, 1 H), 8.68 (s, 1 H), 7.84 (s, 1 H), 7.67–7.61 (m, 1 H), 7.38 (br s, 1 H), 7.28–7.24 (m, 1 H), 7.01 (br s, 1 H), 6.49–6.41 (m, 1 H), 4.84–4.75 (m, 1 H), 3.94–3.86 (m, 1 H), 3.71 (s, 3 H), 3.54–3.50 (m, 1 H), 3.48–3.41 (m, 2 H), 2.33 (s, 3 H), 2.17 (s, 3 H), 2.14 (d, J = 7.3 Hz, 3 H), 1.48 (s, 9 H); % e.e. undetermined; LC-MS (Method A) $[M + H]^+ = 559$, R_t 0.86 min.

8-(2-Aminoethoxy)-7-(3,5-dimethylisoxazol-4-yl)-3-methyl-1-[1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one Hydrochloride (15). A solution of *tert*-butyl [2-({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[(*R*)-1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-

imidazo[4,5-*c*]quinolin-8-yl}oxy)ethyl]carbamate **14** (59 mg, 0.106 mmol) in DCM (4 mL) was treated with TFA (0.5 mL, 6.49 mmol) and the reaction stirred at rt for 90 min. The solvent was removed in vacuo, the residue dissolved in MeOH and applied to a 2 g SCX-2 cartridge. The cartridge was washed with MeOH (10 mL) followed by 2 M ammonia in MeOH solution (10 mL). The basic wash was concentrated to give the title compound as an orange oil (39 mg). 1 M HCl in diethylether (0.047 mmol, 47 µL) was added to 19 mg of the free base and the solvent removed under a stream of nitrogen to give the title compound as a light orange solid (17 mg, 0.033 mmol, 31% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 1 H), 8.68 (d, J = 5.0 Hz, 1 H), 8.16 (br s, 3 H), 8.07 (s, 1 H), 7.90–7.84 (m, 1 H), 7.59 (d, J = 6.8 Hz, 1 H), 7.38 (dd, J = 7.3, 5.0 Hz, 1 H), 7.03 (br s, 1 H), 6.39 (q, J = 7.3 Hz, 1 H), 4.22–4.14 (m, 1 H), 3.64 (s, 3 H), 3.24–3.19 (m, 2 H), 2.31 (s, 3 H), 2.11 (s, 3 H), 2.08 (d, J = 7.3 Hz, 3 H), one OCH_2 proton obscured by water; % e.e. undetermined; LC-MS (Method A) $[M + H]^+$ = 459, R_t 0.50 min.

8-[2-(Dimethylamino)ethoxy]-7-(3,5-dimethylisoxazol-4-yl)-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one Hydrochloride (**16**). 7-(3,5-Dimethylisoxazol-4-yl)-8-hydroxy-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one (50 mg, 0.12 mmol), potassium carbonate (43 mg, 0.31 mmol) and 2-bromo-*N*,*N*-dimethylamine hydrochloride (24 mg, 0.129 mmol) were combined in DMF (5 mL) under nitrogen and heated to 100 ºC for 16 h. The reaction was cooled and evaporated in vacuo to give a black solid which was purified by MDAP (Method A). The appropriate fractions were evaporated under a stream of nitrogen and applied to a 2 g SCX cartridge in the minimum amount of MeOH. The cartridge was washed with MeOH (10 mL) followed by 2 M ammonia in MeOH solution (10 mL) . The basic wash was concentrated in vacuo to give an orange solid which was taken up in the minimum amount of DCM and 1 N HCl in diethyl ether (0.039 mL, 0.039 mmol) added. The solvent was

removed under a stream of nitrogen to give the title compound as a salmon pink solid (17 mg, 0.029 mmol, 24% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.40 (br s, 1 H), 9.06 (s, 1 H), 8.68 (d, J = 4.5 Hz, 1 H), 8.01 (s, 1 H), 7.89–7.81 (m, 1 H), 7.56 (d, J = 7.3 Hz, 1 H), 7.39 (dd, J = 7.3, 4.5 Hz, 1 H), 7.12 (br s, 1 H), 6.38 (q, J = 7.1 Hz, 1 H), 4.37–4.28 (m, 1 H), 3.65–3.62 (m, 3 H), 2.72–2.66 (m, 6 H), 2.30 (s, 3 H), 2.11 (s, 3 H), 2.08 (d, J = 7.1 Hz, 3 H), CH_2 protons obscured by water; % e.e. undetermined; LC-MS (Method A) $[M + H]^+$ = 487, R_t 0.43 min.

***tert*-Butyl [3-({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)propyl]carbamate (17).** A mixture of 7-(3,5-dimethylisoxazol-4-yl)-8-hydroxy-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one **13** (145 mg, 0.349 mmol) and potassium carbonate (60 mg, 0.349 mmol) in DMF (5 mL) and treated with *tert*-butyl (3-bromopropyl)carbamate (102 mg, 0.428 mmol). The mixture was stirred under nitrogen at 100 ºC for 2.5 h and then allowed to cool to rt. EtOAc (10 mL) was added and washed with 10% LiCl (aq) (10 mL). The aqueous layer was extracted with EtOAc (10 mL), the organic layers combined and washed with 10% LiCl (aq) (10 mL). The aqueous layer was extracted with EtOAc (10 mL), the organic layers combined and passed through a hydrophobic frit. The solvent was removed by rotary evaporation. The residue was dissolved in MeOH (1.0 mL) and applied to a MeOH-preconditioned 5 g SCX-2 cartridge. The cartridge was washed with MeOH (30 mL) and 2 M ammonia in MeOH solution (30 mL). The basic wash was evaporated in vacuo and the residue purified by MDAP (Method B) to give the title compound as a brown gum (172 mg, 0.300 mmol, 86% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.76 (s, 1 H), 8.58 (d, J = 4.8 Hz, 1 H), 7.77 (s, 1 H), 7.77–7.72 (m, 1 H), 7.43 (d, J = 8.1 Hz, 1 H), 7.29 (dd, J = 7.3, 4.8 Hz, 1 H), 7.01 (s, 1 H), 6.29 (q, J = 7.3 Hz, 1 H), 6.22 (br s, 1 H), 3.83 (dt, J = 9.9, 6.3 Hz, 1 H), 3.58 (s, 3 H), 3.56 (dt, J = 9.9, 6.3 Hz, 1 H), 2.99 (t, J = 6.3 Hz, 1 H), 2.98 (t, J

= 6.3 Hz, 1 H), 2.25 (s, 3 H), 2.05 (s, 3 H), 2.05 (d, *J* = 7.3 Hz, 3 H), 1.77 (quin, *J* = 6.3 Hz, 2 H), 1.38 (s, 9 H); % e.e. undetermined; LC-MS (Method B) $[M + H]^+$ = 573, R_t 1.07 min.

8-(3-Aminopropoxy)-7-(3,5-dimethylisoxazol-4-yl)-3-methyl-1-[1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one Hydrochloride (18). A solution of *tert*-butyl [3-({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[(*R*)-1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)propyl]carbamate **17** (52.4 mg, 0.092 mmol) in DCM (4 mL) was treated with TFA (1 mL, 12.98 mmol) and the reaction stirred at rt for 90 min. The solvent was removed in vacuo and the residue was applied to a 2 g SCX-2 cartridge in the minimum amount of MeOH. The cartridge was washed with MeOH (10 mL) then 2 M ammonia in MeOH (10 mL). The basic wash was concentrated in vacuo to give the free base as a brown oil (41 mg). 1 M HCl in diethylether (45 µL, 0.045 mmol) was added to 21 mg of the free base and the solvent removed under a stream of nitrogen to give the title compound as a light brown solid (14 mg, 0.025 mmol, 28% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 9.20 (s, 1 H), 8.61 (d, *J* = 4.8 Hz, 1 H), 8.14 (s, 1 H), 7.97 (br s, 3 H), 7.90–7.82 (m, 1 H), 7.60 (d, *J* = 6.8 Hz, 1 H), 7.39 (dd, *J* = 7.3, 4.8 Hz, 1 H), 7.07 (s, 1 H), 6.41 (q, *J* = 7.3 Hz, 1 H), 4.03–3.94 (m, 1 H), 3.64 (s, 3 H), 2.85–2.72 (m, 2 H), 2.32 (s, 3 H), 2.11 (s, 3 H), 2.09 (d, *J* = 7.3 Hz, 3 H), 2.05–1.96 (m, 2 H), one OCH_2 proton obscured by water; % e.e. undetermined; LC-MS (Method A) $[M + H]^+$ = 473, R_t 0.52 min.

7-(3,5-Dimethylisoxazol-4-yl)-8-(3-hydroxypropoxy)-3-methyl-1-[1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one (19). A solution of 7-(3,5-dimethylisoxazol-4-yl)-8-hydroxy-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one **13** (270 mg, 0.65 mmol) in anhydrous DMF (4.5 mL) was dispensed evenly between 6 × vessels each containing potassium carbonate (18 mg, 0.13 mmol). To one of these vessels was added 3-bromo-1-propanol (11 µL, 0.13 mmol) and the mixture stirred under nitrogen at 100 ºC for 16 h. The

reaction mixture was evaporated under a stream of nitrogen and the crude material purified by MDAP (Method B) to give the title compound as a light brown foam (23 mg, 0.05 mmol, 45% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72–8.65 (m, 2 H), 7.84 (s, 1 H), 7.65 (t, J = 7.3 Hz, 1 H), 7.45–7.31 (m, 1 H), 7.27–7.23 (m, 1 H), 7.14–6.91 (m, 1 H), 6.52–6.41 (m, 1 H), 4.09–3.96 (m, 1 H), 3.81–3.70 (m, 6 H), 2.32 (s, 3 H), 2.19–2.15 (m, 6 H), 2.03–1.94 (m, 2 H), O*H* signal not resolved; % e.e. undetermined; LC-MS (Method A) $[M + H]^+$ = 474, R_t 0.64 min.

***N*-[3-({7-(3,5-Dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)propyl]acetamide Hydrochloride (20).** A solution of 8-(3-aminopropoxy)-7-(3,5-dimethylisoxazol-4-yl)-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one **18** (23 mg, 0.048 mmol) in DCM (2 mL) and pyridine (0.25 mL) was treated with acetic anhydride (4.5 µL, 0.048 mmol) and the mixture stirred at rt for 2 h. The reaction was concentrated in vacuo and the residue purified by MDAP (Method A). The residue was dissolved in MeOH and applied to a 2 g SCX-2 cartridge. The cartridge was washed with MeOH followed by 2 M ammonia in MeOH solution. The basic wash was concentrated, dissolved in DCM and treated with 1 M HCl in diethylether (30 µL, 0.030 mmol). The solvent was removed under a stream of nitrogen to give the title compound as an off-white solid (11 mg, 0.019 mmol, 40% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.30 (s, 1 H), 8.57 (d, J = 4.8 Hz, 1 H), 8.13 (s, 1 H), 7.92–7.84 (m, 1 H), 7.66–7.63 (m, 1 H), 7.40–7.37 (m, 2 H), 6.73 (br s, 1 H), 6.43 (q, J = 7.3 Hz, 1 H), 3.87 (m, 1 H), 3.66 (s, 3 H), 3.07 (m, 2 H), 2.32 (s, 3 H), 2.12 (s, 3 H), 2.07 (d, J = 7.3 Hz, 3 H), 1.82–1.75 (m, 5 H), one OCH_2 proton obscured by water; % e.e. undetermined; LC-MS (Method A) $[M + H]^+$ = 515, R_t 0.64 min.

***tert*-Butyl [3-({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)propyl](methyl)carbamate (21).** A solution of

tert-butyl [3-({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)propyl]carbamate **17** (153 mg, 0.267 mmol) in anhydrous NMP (1 mL) under nitrogen was treated with NaH (60% dispersion in mineral oil) (15 mg, 0.374 mmol) and the mixture stirred at rt for 20 min. Methyl iodide (0.018 mL, 0.294 mmol) was added and the mixture was left to stir for 17 h. The mixture was treated with NaH (60% dispersion in mineral oil) (15 mg) and stirred for 20 min before methyl iodide (0.009 mL, 0.147 mmol) was added. After 4.5 h stirring, water (8 mL) was added. The mixture was extracted with EtOAc (2 × 10 mL), the extracts combined and passed through a hydrophobic frit. The solvent was removed by rotary evaporation and the residue purified by MDAP (Method B). The appropriate fractions were combined and the solvent removed by rotary evaporation to give the title compound as a yellow solid (34 mg, 0.058 mmol, 22% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.77 (s, 1 H), 8.58 (d, J = 4.8 Hz, 1 H), 7.78 (s, 1 H), 7.77–7.70 (m, 1 H), 7.42 (d, J = 8.1 Hz, 1 H), 7.29 (dd, J = 7.3, 4.8 Hz, 1 H), 7.02 (s, 1 H), 6.29 (q, J = 7.3 Hz, 1 H), 3.82 (dt, J = 9.9, 6.1 Hz, 1 H), 3.58 (s, 3 H), 3.55 (dt, J = 9.9, 6.1 Hz, 1 H), 3.17 (t, J = 6.9 Hz, 1 H), 3.17 (t, J = 6.9 Hz, 1 H), 2.70 (s, 3 H), 2.25 (s, 3 H), 2.06 (s, 3 H), 2.05 (d, J = 7.3 Hz, 3 H), 1.88–1.79 (m, 2 H), 1.34 (s, 9 H); % e.e. undetermined; LC-MS (Method B) $[M + H]^+$ = 587, R_t 1.13 min.

7-(3,5-Dimethylisoxazol-4-yl)-3-methyl-8-[3-(methylamino)propoxy]-1-[1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one (22). A solution of *tert*-butyl [3-({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)propyl](methyl)carbamate **21** (23 mg, 0.039 mmol) in 1,4-dioxane (0.5 mL) was treated with 4 M HCl in 1,4-dioxane solution (0.5 mL, 0.039 mmol) and the mixture stirred at rt for 2 h in a stoppered vessel. The reaction mixture was evaporated under a stream of nitrogen and the residue was dissolved in MeOH (0.5 mL). The solution was applied to a MeOH-

preconditioned 2 g SCX-2 cartridge. The cartridge was washed with MeOH (16 mL) followed by 2 M ammonia in MeOH solution (16 mL). The basic wash was evaporated under in vacuo to give a light brown oil which solidified upon standing (19 mg, 0.039 mmol, 100% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.76 (s, 1 H), 8.59 (d, J = 4.8 Hz, 1 H), 7.78 (s, 1 H), 7.77–7.71 (m, 1 H), 7.41 (d, J = 8.1 Hz, 1 H), 7.29 (dd, J = 7.3, 4.8 Hz, 1 H), 7.02 (s, 1 H), 6.29 (q, J = 7.3 Hz, 1 H), 3.86 (dt, J = 9.7, 6.4 Hz, 1 H), 3.58 (s, 3 H), 3.63–3.57 (m, 1 H), 2.52 (t, J = 6.7 Hz, 2 H), 2.29 (s, 3 H), 2.24 (s, 3 H), 2.05 (s, 3 H), 2.05 (d, J = 7.1 Hz, 3 H), 1.74 (tt, J = 6.7, 6.4 Hz, 2 H), N*H* signal not resolved; % e.e. undetermined; LC-MS (Method B) $[M + H]^+$ = 487, R_t 0.77 min.

8-[3-(Dimethylamino)propoxy]-7-(3,5-dimethylisoxazol-4-yl)-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one (23). A mixture of 7-(3,5-dimethylisoxazol-4-yl)-8-hydroxy-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one **13** (45 mg, 0.108 mmol), potassium carbonate (36 mg, 0.260 mmol) and 3-bromo-*N*,*N*-dimethylpropan-1-amine hydrobromide (32 mg, 0.130 mmol) was suspended in anhydrous DMF (0.8 mL) and stirred under nitrogen at 100 ºC for 2 h. The reaction mixture was allowed to cool to rt, diluted with MeOH (0.2 mL) and filtered. The solution was purified by MDAP (Method B). The appropriate fractions were combined and the solvent removed by rotary evaporation to give the title compound as an off-white solid (34 mg, 0.068 mmol, 63% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.78 (s, 1 H), 8.61 (d, J = 4.8 Hz, 1 H), 7.81 (s, 1 H), 7.79–7.73 (m, 1 H), 7.44 (d, J = 8.1 Hz, 1 H), 7.31 (dd, J = 7.3, 4.8 Hz, 1 H), 7.09 (s, 1 H), 6.33 (q, J = 7.3 Hz, 1 H), 3.87 (dt, J = 9.9, 6.1 Hz, 1 H), 3.67–3.59 (m, 4 H), 2.30–2.24 (m, 5 H), 2.16 (s, 6 H), 2.11–2.07 (m, 6 H), 1.80–1.71 (m, 2 H); % e.e. undetermined; LC-MS (Method A) $[M + H]^+$ = 501, R_t 0.55 min.

***tert*-Butyl 2-({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)acetate (24).** A mixture of 7-(3,5-

dimethylisoxazol-4-yl)-8-hydroxy-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one **13** (60 mg, 0.14 mmol) and potassium carbonate (24 mg, 0.17 mmol) was suspended in anhydrous DMF (1 mL) and treated with *tert*-butyl bromoacetate (0.026 mL, 0.17 mmol). The mixture was stirred under nitrogen at 100 ºC for 5 h. The reaction mixture was allowed to cool to rt and partially evaporated under a stream of nitrogen (~ 0.9 mL remaining). The mixture was filtered and purified by MDAP (Method A). The appropriate fractions were combined and the solvent removed by rotary evaporation. The solid was dissolved in MeOH (0.5 mL) and applied to a MeOH-preconditioned 1 g SCX-2 cartridge. The cartridge was washed with MeOH (3 mL) followed by 2 M ammonia in MeOH solution (3 mL). The basic wash was evaporated under a stream of nitrogen to give the title compound as a light brown solid (37 mg, 0.07 mmol, 48% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.80 (s, 1 H), 8.62 (d, J = 4.8 Hz, 1 H), 7.85 (s, 1 H), 7.80–7.74 (m, 1 H), 7.46 (d, J = 8.1 Hz, 1 H), 7.33 (dd, J = 7.3, 4.8 Hz, 1 H), 7.15 (s, 1 H), 6.29 (q, J = 7.2 Hz, 1 H), 4.43–4.31 (m, 2 H), 3.59 (s, 3 H), 2.30 (s, 3 H), 2.13 (s, 3 H), 2.06 (d, J = 7.2 Hz, 3 H), 1.47 (s, 9 H); % e.e. undetermined; LC-MS (Method A) $[M + H]^+$ = 530, R_t 0.96 min.

2-({7-(3,5-Dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[(*R*)-1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)acetic acid (25). A solution of *tert*-butyl 2-({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)acetate **24** (29 mg, 0.06 mmol) in DCM (0.75 mL) was treated with TFA (0.5 mL, 6.49 mmol) and left to stand in a stoppered vessel for 2 h. The reaction mixture was evaporated in vacuo. The solid was dissolved in MeOH (0.5 mL) and applied to 0.5 g MeOH-preconditioned SCX-2 cartridge. The cartridge was washed with MeOH (3 mL) followed by 2 M ammonia in MeOH solution (3 mL). The basic wash was evaporated under a stream of nitrogen to

give the title compound as an off-white solid (15 mg, 0.032 mmol, 58% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) 8.79 (s, 1 H), 8.60 (d, J = 4.8 Hz, 1 H), 7.83 (s, 1 H), 7.80–7.73 (m, 1 H), 7.46 (d, J = 8.1 Hz, 1 H), 7.30 (dd, J = 7.3, 4.8 Hz, 1H), 7.15 (s, 1 H), 6.31 (q, J = 7.2 Hz, 1 H), 4.44–4.34 (m, 2 H), 3.60 (s, 3 H), 2.31 (s, 3 H), 2.14 (s, 3 H), 2.07 (d, J = 7.2 Hz, 3 H), CO_2H signal not resolved; % e.e. undetermined; LC-MS (Method A) [M + H$^+$] = 474, R_t 0.61 min, 94% purity by LC-MS UV.

***tert*-Butyl [*cis*-3-({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)cyclobutyl]carbamate (26).** A mixture of *trans*-3-[(*tert*-butoxycarbonyl)amino]cyclobutyl methanesulfonate (281 mg, 1.06 mmol), potassium carbonate (160 mg, 1.16 mmol) and 7-(3,5-dimethylisoxazol-4-yl)-8-hydroxy-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one **13** (400 mg, 0.96 mmol) was suspended in anhydrous DMF (4 mL) and stirred under nitrogen at 100 ºC for 6 h. The reaction mixture was allowed to cool to rt and diluted with brine (20 mL). The mixture was extracted with DCM (2 × 20 mL), the organic layers combined and passed through a hydrophobic frit. The solvent was removed in vacuo to give a brown oil. The oil was dissolved in MeOH (5 mL) and applied to a MeOH-preconditioned 50 g SCX-2 cartridge. The cartridge was washed with MeOH (200 mL) followed by 2 M ammonia in MeOH solution (200 mL). The basic fraction was evaporated in vacuo to give a brown gum. The crude material was purified by MDAP (Method A). The appropriate fractions were combined and the solvent removed by rotary evaporation to give the title compound as a white solid (233 mg, 0.40 mmol, 41% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.77 (s, 1 H), 8.63 (d, J = 4.8 Hz, 1 H), 7.80 (s, 1 H), 7.79–7.72 (m, 1 H), 7.42 (d, J = 7.8 Hz, 1 H), 7.33 (dd, J = 7.8, 4.8 Hz, 1 H), 6.89 (s, 1 H), 6.64 (d, J = 6.3 Hz, 1 H), 6.27 (q, J = 7.2 Hz, 1 H), 3.92–3.83 (m, 1 H), 3.70–3.60 (m, 1 H), 3.58 (s, 3 H), 2.82–2.72 (m, 1 H), 2.54–2.44

(m, 1 H), 2.27 (s, 3 H), 2.09 (s, 3 H), 2.06 (d, J = 7.2 Hz, 3 H), 1.94–1.84 (m, 2 H), 1.40 (s, 9 H); % e.e. undetermined; LC-MS (Method A) $[M + H]^+$ = 585, R_t 0.96 min.

8-(*cis*-3-Aminocyclobutoxy)-7-(3,5-dimethylisoxazol-4-yl)-3-methyl-1-[1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one (27). A solution of *tert*-butyl [*cis*-3-({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)cyclobutyl]carbamate **26** (229 mg, 0.39 mmol) in DCM (6 mL) was treated with TFA (2 mL, 26.0 mmol). The mixture was allowed to stand in a stoppered vessel at rt for 2 h. The reaction mixture was evaporated under a stream of nitrogen and the residue dissolved in MeOH (2 mL). The solution was applied to a MeOH-preconditioned 5 g SCX-2 cartridge. The cartridge was washed with MeOH (30 mL) followed by 2 M ammonia in MeOH solution (30 mL). The basic wash was evaporated under a stream of nitrogen to give the title compound as an off-white solid (178 mg, 0.37 mmol, 94% yield). mp 158–160 ºC; ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.77 (s, 1 H), 8.62 (d, J = 4.8 Hz, 1 H), 7.80 (s, 1 H), 7.78–7.71 (m, 1 H), 7.41 (d, J = 7.8 Hz, 1 H), 7.32 (dd, J = 7.8, 4.8 Hz, 1 H), 6.87 (s, 1 H), 6.27 (q, J = 7.3 Hz, 1 H), 3.83–3.75 (m, 1 H), 3.58 (s, 3 H), 3.09–3.00 (m, 1 H), 2.80–2.71 (m, 1 H), 2.52–2.44 (m, 1 H), 2.27 (s, 3 H), 2.09 (s, 3 H), 2.06 (d, J = 7.3 Hz, 3 H), 1.65–1.54 (m, 2 H), NH_2 signal not resolved; ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.0, 159.1, 158.9, 153.7, 152.4, 149.3, 140.1, 137.5, 133.1, 131.6, 127.1, 124.0, 122.8, 120.8, 120.6, 115.2, 112.1, 101.6, 64.7, 52.7, 41.2, 40.4, 39.7, 27.7, 17.5, 11.5, 10.5; % e.e. undetermined; LC-MS (Method A) $[M + H]^+$ = 485, R_t 0.52 min; HRMS $[M + H]^+$ calcd for $C_{27}H_{29}N_6O_3$ 485.2301, found 485.2292.

***tert*-Butyl [*trans*-3-({7-(3,5-dimethyl-4-isoxazolyl)-3-methyl-2-oxo-1-[1-(pyridine-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)cyclobutyl]carbamate (28).** A mixture of *cis*-3-[(*tert*-butoxycarbonyl)amino]cyclobutyl methanesulfonate (63 mg, 0.24 mmol),

potassium carbonate (36 mg, 0.26 mmol) and 7-(3,5-dimethylisoxazol-4-yl)-8-hydroxy-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one **13** (90 mg, 0.22 mmol) was suspended in anhydrous DMF (1 mL) and stirred under nitrogen at rt overnight. The temperature was raised to 100 ºC and the reaction left to stir for 6 h. The reaction mixture was allowed to cool to rt and filtered. The filtrate was purified by MDAP (Method B). The appropriate fractions were combined and the solvent removed by rotary evaporation to give the title compound as a light brown gum (84 mg, 0.14 mmol, 66% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.77 (s, 1 H), 8.65 (d, J = 4.8 Hz, 1 H), 7.81 (s, 1 H), 7.78–7.70 (m, 1 H), 7.39 (d, J = 7.8 Hz, 1 H), 7.30 (dd, J = 7.8, 4.8 Hz, 1 H), 6.80 (s, 1 H), 6.69 (d, J = 5.6 Hz, 1 H), 6.26 (q, J = 7.3 Hz, 1 H), 4.36–4.27 (m, 1 H), 4.06–3.95 (m, 1 H), 3.57 (s, 3 H), 2.51–2.42 (m, 1 H), 2.27 (s, 3 H), 2.32–2.08 (m, 3 H), 2.09 (s, 3 H), 2.05 (d, J = 7.3 Hz, 3 H), 1.41 (s, 9 H); % e.e. undetermined; LC-MS (Method A) $[M + H]^+$ = 585, R_t 0.93 min.

8-(*trans*-3-Aminocyclobutoxy)-7-(3,5-dimethylisoxazol-4-yl)-3-methyl-1-[1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one (29). A solution of *tert*-butyl [*trans*-3-({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[(*R*)-1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)cyclobutyl]carbamate **28** (75 mg, 0.13 mmol) in DCM (1.5 mL) was treated with TFA (0.5 mL, 6.49 mmol). The mixture was allowed to stand in a stoppered vessel at rt for 2 h. The reaction mixture was evaporated under a stream of nitrogen and the crude material purified by MDAP (Method B). The appropriate fractions were combined and the solvent removed by rotary evaporation to give the title compound as an off-white solid (48 mg, 0.10 mmol, 77% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.76 (s, 1 H) 8.68 (d, J = 4.8 Hz, 1 H), 7.80 (s, 1 H), 7.78–7.71 (m, 1 H), 7.40 (d, J = 7.8 Hz, 1 H), 7.31 (dd, J = 7.8, 4.8 Hz, 1 H), 6.81 (s, 1 H), 6.27 (q, J = 7.3 Hz, 1 H), 4.38–4.31 (m, 1 H), 3.58 (s, 3 H), 3.56–3.49 (m, 1 H), 2.27 (s, 3 H),

2.18–2.13 (m, 2 H), 2.08 (s, 3 H), 2.06 (d, J = 7.3 Hz, 3 H), 2.12–2.03 (m, 1 H), 1.99–1.91 (m, 1 H), 1.73 (br s, 2 H); % e.e. undetermined; LC-MS (Method A) $[M + H]^+$ = 485, R_t 0.52 min.

(*S*)-*tert*-Butyl 3-[(methylsulfonyl)oxy]pyrrolidine-1-carboxylate. A stirred solution of (*S*)-*tert*-butyl 3-hydroxypyrrolidine-1-carboxylate (500 mg, 2.67 mmol) and triethylamine (0.931 mL, 6.68 mmol) in DCM (6 mL) at 0 ºC was treated with methanesulfonyl chloride (0.250 mL, 3.20 mmol). The mixture was stirred under nitrogen for 10 min and allowed to warm to rt over 2 h. The reaction was diluted with DCM (10 mL) and washed with brine (15 mL). The aqueous layer was extracted with DCM (10 mL), the organic layers combined and passed through a hydrophobic frit. The solvent was removed by rotary evaporation and the crude material purified on a 25 g silica cartridge using a gradient of 0–15% MeOH in DCM over 10 column volumes. The appropriate fractions were combined and the solvent was removed by rotary evaporation to give the title compound as a light yellow oil (691 mg, 2.60 mmol, 98% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.28–5.23 (m, 1 H), 3.75–3.41 (m, 4 H), 3.04 (s, 3 H), 2.37–2.08 (m, 2 H), 1.45 (s, 9 H).

(3*R*)-*tert*-Butyl 3-({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[(*R*)-1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)pyrrolidine-1-carboxylate (30). To a vessel containing (*S*)-*tert*-butyl 3-[(methylsulfonyl)oxy]pyrrolidine-1-carboxylate (89 mg, 0.34 mmol) was added potassium carbonate (47 mg, 0.34 mmol) and 7-(3,5-dimethylisoxazol-4-yl)-8-hydroxy-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one **13** (103 mg, 0.25 mmol). DMF (2 mL) was added and the mixture stirred under nitrogen at 100 ºC for 4 h. EtOAc (10 mL) was added to the reaction mixture which was then washed with brine (10 mL). The aqueous layer was extracted with EtOAc (10 mL), the organic layers combined and passed through a hydrophobic frit. The solvent was removed by rotary evaporation and the crude material purified on a 25 g silica cartridge using a gradient of 0–15% MeOH in DCM over 10

column volumes. The appropriate fractions were combined and the solvent removed by rotary evaporation. The sample was dissolved in EtOH (3 mL) and heptane (3 mL). Fifteen injections were made onto a Chiralpak® IA column (250 mm x 30 mm, 5 μm packing diameter). An isocratic system of 15% EtOH in heptane with a flow rate of 40 mL/min was used at rt The UV detection was performed at 215 nm. The appropriate fractions were combined and evaporated in vacuo to give the title compound (49 mg, 0.08 mmol, 34% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.79 (s, 1 H), 8.60 (d, J = 4.8 Hz, 1 H), 7.81 (s, 1 H), 7.78–7.72 (m, 1 H), 7.44 (d, J = 7.8 Hz, 1 H), 7.30 (dd, J = 7.3, 4.8 Hz, 1 H), 7.09 (s, 1 H), 6.29 (q, J = 7.3 Hz, 1 H), 4.55–4.50 (m, 1 H), 3.58 (s, 3 H), 3.38–3.29 (m, 2 H), 3.19–3.11 (m, 2 H), 2.22 (s, 3 H), 2.17–2.08 (m, 1 H), 2.06 (d, J = 7.3 Hz, 3 H), 2.02 (s, 3 H), 1.98–1.89 (m, 1 H), 1.39 (s, 9 H), only a single diasteroisomer was observed; LC-MS (Method B) $[M + H]^+$ = 585, R_t 1.11 min.

7-(3,5-Dimethylisoxazol-4-yl)-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-8-[(*R*)-pyrrolidin-3-yloxy]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one (31). A solution of (3*R*)-*tert*-butyl 3-({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)pyrrolidine-1-carboxylate **30** (45 mg, 0.08 mmol) in 1,4-dioxane (0.5 mL) was treated with 4 M HCl in 1,4-dioxane solution (0.5 mL, 16.46 mmol). The mixture was left to stir at rt for 2 h. The reaction mixture was evaporated under a stream of nitrogen and the solid dissolved in MeOH. The solution was applied to a MeOH-preconditioned 2 g SCX-2 cartridge which was then washed with MeOH (12 mL) followed by 2 M ammonia in MeOH solution (12 mL). The basic wash was evaporated by rotary evaporation to give the title compound as a light brown solid (31 mg, 0.06 mmol, 83% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.77 (s, 1 H), 8.60 (d, J = 4.8 Hz, 1 H), 7.80 (s, 1 H), 7.78–7.72 (m, 1 H), 7.41 (d, J = 7.8 Hz, 1 H), 7.30 (dd, J = 7.3, 4.8 Hz, 1 H), 7.04 (s, 1 H), 6.29 (q, J = 7.3 Hz, 1 H), 4.42–4.35 (m, 1 H), 3.58 (s, 3 H),

2.98–2.92 (m, 2 H), 2.68–2.62 (m, 1 H), 2.24 (s, 3 H), 2.07 (d, J = 7.3 Hz, 3 H), 2.05 (s, 3 H), 2.05–1.99 (m, 1 H), 1.72–1.64 (m, 1 H), one pyrrolidine proton signal obscured by water, N*H* signal not resolved, only a single diasteroisomer was observed; LC-MS (Method B) $[M + H]^+$ = 485, R_t 0.75 min.

(*R*)-*tert*-Butyl 3-[(methylsulfonyl)oxy]pyrrolidine-1-carboxylate. A stirred solution of (*R*)-*tert*-butyl 3-hydroxypyrrolidine-1-carboxylate (481 mg, 2.57 mmol) and triethylamine (0.931 mL, 6.68 mmol) in DCM (6 mL) at 0 ºC was treated with methanesulfonyl chloride (0.250 mL, 3.20 mmol). The mixture was stirred under nitrogen for 10 min and allowed to warm to rt over 1 h. The reaction was diluted with DCM (10 mL) and washed with water (10 mL). The aqueous layer was extracted with DCM (10 mL), the organic layers combined and passed through a hydrophobic frit. The solvent was removed by rotary evaporation and the crude material purified on a 25 g silica cartridge using a gradient of 0–15% MeOH in DCM over 10 column volumes. The appropriate fractions were combined and the solvent was removed by rotary evaporation to give the title compound as a light yellow oil (651 mg, 2.45 mmol, 96% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.28–5.23 (m, 1 H), 3.75–3.41 (m, 4 H), 3.04 (s, 3 H), 2.37–2.08 (m, 2 H), 1.45 (s, 9 H).

(3*S*)-*tert*-Butyl 3-({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)pyrrolidine-1-carboxylate (32). To a vessel containing (*R*)-*tert*-butyl 3-[(methylsulfonyl)oxy]pyrrolidine-1-carboxylate (82 mg, 0.31 mmol) was added potassium carbonate (43 mg, 0.31 mmol) and 7-(3,5-dimethylisoxazol-4-yl)-8-hydroxy-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one **13** (100 mg, 0.24 mmol). DMF (2 mL) was added and the mixture stirred under nitrogen and at 100 ºC for 5 h. EtOAc (10 mL) was added to the reaction mixture which was then washed with brine (10 mL). The aqueous layer was extracted with EtOAc, the organic layers combined and passed through a

hydrophobic frit. The solvent was removed by rotary evaporation and the crude product dissolved in DCM (4 mL) and purified on a 25 g silica cartridge using a gradient of 0–15% MeOH in DCM over 10 column volumes. The appropriate fractions were combined and the solvent removed by rotary evaporation. The crude material was purified by MDAP (Method A). The appropriate fractions were combined and the solvent removed by rotary evaporation to give the title compound as an off-white solid (99 mg, 0.17 mmol, 70% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.80 (s, 1 H), 8.60 (d, J = 4.8 Hz, 1 H), 7.82 (s, 1 H), 7.79–7.73 (m, 1 H), 7.45 (d, J = 7.3 Hz, 1 H), 7.31 (dd, J = 7.3, 4.8 Hz, 1 H), 7.06 (s, 1 H), 6.30 (q, J = 7.3 Hz, 1 H), 4.47 (m, 1 H), 3.59 (s, 3 H), 3.58–3.52 (m, 1 H), 3.39–3.30 (m, 2 H), 3.19–3.11 (m, 1 H), 2.22 (s, 3 H), 2.06 (d, J = 7.3 Hz, 3 H), 2.02 (s, 3 H), 2.01–1.94 (m, 1 H), 1.82–1.73 (m, 1 H), 1.42 (s, 1.3 H), 1.39 (s, 7.7 H), a 85:15 mixture of diastereoisomers present; LC-MS (Method A) $[M + H]^+$ = 585, R_t 0.96 min.

7-(3,5-Dimethylisoxazol-4-yl)-3-methyl-1-[1-(pyridin-2-yl)ethyl]-8-[(*S*)-pyrrolidin-3-yloxy]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one (33). A solution of (3*S*)-*tert*-butyl 3-({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)pyrrolidine-1-carboxylate **32** (103 mg, 0.18 mmol) in anhydrous 1,4-dioxane (0.5 mL) was treated with 4 M HCl in 1,4-dioxane solution (0.5 mL, 16.46 mmol) and the mixture allowed to stand in a stoppered vessel for 2 h. The reaction mixture was evaporated under a stream of nitrogen and the solid dissolved in MeOH. The solution was applied to a MeOH-preconditioned 2 g SCX-2 cartridge. The cartridge was washed with MeOH (20 mL) followed by 2 M ammonia in MeOH solution (20 mL). The basic wash was evaporated by rotary evaporation. Starting material still remained so the residue was dissolved in anhydrous 1,4-dioxane (0.5 mL) and 4 M HCl in 1,4-dioxane solution (0.5 mL, 16.46 mmol), and the mixture left to stir overnight in a stoppered vessel. The reaction mixture was evaporated under a stream of nitrogen and the solid

was dissolved in the minimum volume of MeOH. The solution was applied to a MeOH-preconditioned 2 g SCX-2 cartridge which was then washed with MeOH (20 mL) followed by 2 M ammonia in MeOH solution (20 mL). The basic wash was evaporated by rotary evaporation and the crude material purified by MDAP conducted on an Xbridge BEH Shield RP18 column (150 mm x 19 mm, 5 µm packing diameter) at 20 mL/min flow rate. Gradient elution was carried out at ambient temperature, with the mobile phases as (A) water containing 0.1% (v/v) formic acid and (B) acetonitrile containing 0.1% (v/v) formic acid. The UV detection was a summed signal from wavelength of 210 nm to 400 nm. The appropriate fractions were combined and dried under a stream of nitrogen and the crude material purified on a 10 g silica cartridge using a gradient of 0–20% 2 M ammonia/MeOH in DCM over 15 column volumes. The appropriate fractions were combined and evaporated in vacuo to give the title compound as a colorless oil (10 mg, 0.02 mmol, 12% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.77 (s, 1 H), 8.60 (d, J = 4.8 Hz, 1 H), 7.79 (s, 1 H), 7.77–7.71 (m, 1 H), 7.41 (d, J = 7.3 Hz, 1 H), 7.31 (dd, J = 7.3, 4.8 Hz, 1 H), 7.01 (s, 1 H), 6.29 (q, J = 7.3 Hz, 1 H), 4.37 (m, 1 H), 3.58 (s, 3 H), 3.21–3.15 (m, 0.15 H), 3.13–3.07 (m, 0.85 H), 2.80–2.62 (m, 3 H), 2.24 (s, 2.6 H), 2.19 (s, 0.4 H), 2.10–2.02 (m, 5.1 H), 2.01 (s, 0.9 H), 1.82–1.71 (m, 1 H), 1.55–1.47 (m, 1 H), a 85:15 mixture of diastereoisomers present; LC-MS (Method A) $[M + H]^+$ = 485, R_t 0.52 min.

(*R*)-*tert*-Butyl 3-{[(methylsulfonyl)oxy]methyl}pyrrolidine-1-carboxylate. To a solution of (*R*)-*tert*-butyl 3-(hydroxymethyl)pyrrolidine-1-carboxylate (162 mg, 0.81 mmol) in DCM (3 mL) was added triethylamine (0.14 mL, 1.01 mmol). This was cooled to 0 ºC under a nitrogen atmosphere and methanesulfonyl chloride (0.08 mL, 1.03 mmol) added dropwise. The mixture was stirred at 0 ºC for 10 min, allowed to warm to rt, and then stirred for 2 h. Water (10 mL) was added to the reaction mixture which was then extracted with DCM (2 × 10 mL). The combined

organic layers were passed through a hydrophobic frit and the solvent evaporated in vacuo. The crude material was purified on a 25 g silica cartridge using a gradient of 0–10% MeOH in DCM over 10 column volumes. The appropriate fractions were combined and the solvent removed by rotary evaporation to give the title compound as a colorless oil (204 mg, 0.73 mmol, 91% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.28–4.11 (m, 2 H), 3.62–3.09 (m, 4 H), 3.03 (s, 3 H), 2.70–2.56 (m, 1 H), 2.11–1.99 (m, 1 H), 1.73 (br s, 1 H), 1.46 (s, 9 H).

(3*R*)-*tert*-Butyl 3-[({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)methyl]pyrrolidine-1-carboxylate (34). A mixture of (*R*)-*tert*-butyl 3-{[(methylsulfonyl)oxy]methyl}pyrrolidine-1-carboxylate (52 mg, 0.19 mmol), potassium carbonate (28 mg, 0.20 mmol) and 7-(3,5-dimethylisoxazol-4-yl)-8-hydroxy-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one **13** (70 mg, 0.17 mmol) was suspended in anhydrous DMF (0.8 mL) and stirred under nitrogen at 45 ºC for 16 h. The reaction temperature was raised to 100 ºC and left to stir for 3.5 h. The reaction mixture was allowed to cool to rt and diluted with MeOH (0.2 mL). The mixture was filtered and purified by MDAP (Method A). The appropriate fractions were combined and the solvent removed by rotary evaporation to give the title compound as a dark brown gum (79 mg, 0.13 mmol, 78% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.81 (s, 1 H), 8.59 (d, J = 4.8 Hz, 1 H), 7.82 (s, 1 H), 7.80–7.73 (m, 1 H), 7.46 (d, J = 7.8 Hz, 1 H), 7.32 (dd, J = 7.3, 4.8 Hz, 1 H), 7.03 (s, 0.15 H), 7.01 (s, 0.85 H), 6.32 (q, J = 7.1 Hz, 1 H), 3.81 (dd, J = 9.6, 6.1 Hz, 0.15 H), 3.76 (dd, J = 9.5, 7.2 Hz, 0.85 H), 3.62 (s, 3 H), 3.51–3.42 (m, 1 H), 3.42–3.35 (m, 1 H), 3.35–3.22 (m, 2 H), 3.03–2.98 (m, 1 H), 2.55–2.47 (m, 1 H), 2.26 (s, 3 H), 2.07 (s, 3 H), 2.07 (d, J = 7.1 Hz, 3 H), 1.99–1.88 (m, 1 H), 1.64–1.53 (m, 1 H), 1.45 (s, 9 H), a 85:15 mixture of diastereoisomers present; LC-MS (Method A) $[M + H]^+$ = 599, R_t 0.98 min.

7-(3,5-Dimethylisoxazol-4-yl)-3-methyl-1-[1-(pyridin-2-yl)ethyl]-8-[(*R*)-pyrrolidin-3-ylmethoxy]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one (35). A solution of (3*R*)-*tert*-butyl 3-[({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[(*R*)-1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)methyl]pyrrolidine-1-carboxylate **34** (73 mg, 0.12 mmol) was dissolved in DCM (1.5 mL) and treated with TFA (0.5 mL, 6.49 mmol). The mixture was allowed to stand in a stoppered vessel at rt for 2 h. The reaction mixture was evaporated in vacuo and the crude material purified by MDAP (Method B). The appropriate fractions were combined and the solvent removed by rotary evaporation to give the title compound as a white solid (44 mg, 0.09 mmol, 72% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.77 (s, 1 H), 8.58 (d, J = 4.8 Hz, 1 H), 7.78 (s, 1 H), 7.77–7.72 (m, 1 H), 7.42 (d, J = 7.3 Hz, 1 H), 7.30 (dd, J = 7.3, 4.8 Hz, 1 H), 6.99 (s, 1 H), 6.29 (q, J = 7.3 Hz, 1 H), 3.74 (dd, J = 9.8, 3.2 Hz, 0.15 H), 3.69 (dd, J = 9.6, 6.3 Hz, 0.85 H), 3.59 (s, 3 H), 3.46–3.37 (m, 1 H), 2.84–2.78 (m, 2 H), 2.53–2.49 (m, 1 H), 2.38–2.27 (m, 1 H), 2.24 (s, 3 H), 2.04 (s, 3 H), 2.05 (d, J = 7.3 Hz, 3 H), 1.84–1.74 (m, 1 H), 1.40–1.28 (m, 1 H), one pyrrolidine CH_2N proton signal obscured by water, N*H* signal not resolved, a 85:15 mixture of diastereoisomers present; LC-MS (Method A) $[M + H]^+$ = 499, R_t 0.53 min.

(*S*)-*tert*-Butyl 3-{[(methylsulfonyl)oxy]methyl}pyrrolidine-1-carboxylate. To a solution of (*S*)-*tert*-butyl 3-(hydroxymethyl)pyrrolidine-1-carboxylate (174 mg, 0.87 mmol) in DCM (3 mL) was added triethylamine (0.150 mL, 1.08 mmol). This was cooled to 0 ºC under a nitrogen atmosphere and methanesulfonyl chloride (0.086 mL, 1.10 mmol) added dropwise. The mixture was stirred at 0 ºC for 10 min, allowed to warm to rt, and then stirred for 2 h. Water (10 mL) was added to the reaction mixture which was then extracted with DCM (2 × 10 mL). The combined organic layers were passed through a hydrophobic frit and the solvent evaporated in vacuo. The crude material was purified on a 25 g silica cartridge using a gradient of 0–10% MeOH in DCM

over 10 column volumes. The appropriate fractions were combined and the solvent removed by rotary evaporation to give the title compound as a colorless oil (219 mg, 0.78 mmol, 91% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.28–4.11 (m, 2 H), 3.62–3.09 (m, 4 H), 3.03 (s, 3 H), 2.70–2.56 (m, 1 H), 2.11–1.99 (m, 1 H), 1.73 (br s, 1 H), 1.46 (s, 9 H).

(3*S*)-*tert*-Butyl 3-[({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)methyl]pyrrolidine-1-carboxylate (36). A mixture of (*S*)-*tert*-butyl 3-{[(methylsulfonyl)oxy]methyl}pyrrolidine-1-carboxylate (37 mg, 0.13 mmol), potassium carbonate (20 mg, 0.14 mmol) and 7-(3,5-dimethylisoxazol-4-yl)-8-hydroxy-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one **13** (50 mg, 0.12 mmol) was suspended in anhydrous DMF (0.5 mL) and stirred under nitrogen at 100 ºC for 4 h. The reaction mixture was allowed to cool to rt and diluted with MeOH (0.5 mL). The mixture was filtered and was purified by MDAP (Method B). The appropriate fractions were combined and the solvent removed by rotary evaporation to give the title compound as a colorless gum (49 mg, 0.08 mmol, 68% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.77 (s, 1 H), 8.57 (d, J = 4.8 Hz, 1 H), 7.79 (s, 1 H), 7.76–7.70 (m, 1 H), 7.42 (d, J = 7.8 Hz, 1 H), 7.29 (dd, J = 7.3, 4.8 Hz, 1 H), 7.02 (s, 0.85 H), 7.00 (s, 0.15 H), 6.29 (q, J = 7.1 Hz, 1 H), 3.79 (dd, J = 9.6, 6.3 Hz, 0.85 H), 3.73 (dd, J = 9.6, 7.0 Hz, 0.15 H), 3.59 (s, 3 H), 3.45–3.39 (m, 1 H), 3.37–3.28 (m, 2 H), 3.26–3.19 (m, 1 H), 2.94 (dd, J = 10.9, 6.8 Hz, 1 H), 2.52–2.44 (m, 1 H), 2.23 (s, 3 H), 2.05 (d, J = 7.1 Hz, 3 H), 2.04 (s, 3 H), 1.98–1.87 (m, 1 H), 1.62–1.52 (m, 1 H), 1.41 (s, 9 H), a 85:15 mixture of diastereoisomers present; LC-MS (Method A) $[M + H]^+$ = 599, R_t 0.94 min.

7-(3,5-Dimethylisoxazol-4-yl)-3-methyl-1-[1-(pyridin-2-yl)ethyl]-8-[(*S*)-pyrrolidin-3-ylmethoxy]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one (37). A solution of (3*S*)-*tert*-butyl 3-[({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[(*R*)-1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-

imidazo[4,5-*c*]quinolin-8-yl}oxy)methyl]pyrrolidine-1-carboxylate **36** (39 mg, 0.07 mmol) was dissolved in DCM (0.6 mL) and treated with TFA (0.3 mL, 3.89 mmol). The mixture was allowed to stand in a stoppered vessel at rt for 2 h. The reaction mixture was evaporated under a stream of nitrogen and the gum dissolved in MeOH (0.5 mL). The solution was applied to a MeOH-preconditioned 1 g SCX-2 cartridge which was then washed with MeOH (6 mL) followed by 2 M ammonia in MeOH solution (6 mL). The basic wash was evaporated under a stream of nitrogen to give the title compound as a white solid (28 mg, 0.06 mmol, 86% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.77 (s, 1 H), 8.58 (d, J = 4.8 Hz, 1 H), 7.78 (s, 1 H), 7.77–7.71 (m, 1 H), 7.42 (d, J = 7.8 Hz, 1 H), 7.30 (dd, J = 7.3, 4.8 Hz, 1 H), 6.98 (s, 1 H), 6.29 (q, J = 7.1 Hz, 1 H), 3.74–3.65 (m, 1 H), 3.59 (s, 3 H), 3.40–3.34 (m, 1 H), 2.79–2.70 (m, 4 H), 2.42 (dd, J = 10.6, 6.3 Hz, 1 H), 2.34–2.25 (m, 1 H), 2.24 (s, 3 H), 2.04 (s, 3 H), 2.05 (d, J = 7.1 Hz, 3 H), 1.82–1.71 (m, 1 H), 1.36–1.26 (m, 1 H), one pyrrolidine proton signal obscured by water, N*H* signal not resolved, an estimated 85:15 mixture of diastereoisomers present; LC-MS (Method A) $[M + H]^+$ = 499, R_t 0.53 min.

***tert*-Butyl 4-{[(methylsulfonyl)oxy]methyl}piperidine-1-carboxylate.** A stirred solution of *tert*-butyl 4-(hydroxymethyl)piperidine-1-carboxylate (310 mg, 1.440 mmol) and triethylamine (0.26 mL, 1.865 mmol) in DCM (3 mL) at 0 ºC was treated with methanesulfonyl chloride (0.13 mL, 1.668 mmol). The mixture was stirred for 1 h under nitrogen and then sat. $NaHCO_3$ (aq) (10 mL) was added. The aqueous layer was separated and extracted with DCM (10 mL). The organic layers were combined and washed with sat. $NaHCO_3$ (aq) (10 mL). The organic layer was separated, passed through a hydrophobic frit and the solvent evaporated under vacuum to give the title compound as a yellow oil (386 mg, 1.316 mmol, 91% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ

4.23–4.10 (m, 2 H), 4.10–4.04 (m, 2 H), 3.01 (s, 3 H), 2.78–2.65 (m, 2 H), 1.98–1.86 (m, 1 H), 1.79–1.69 (m, 2 H), 1.46 (br s, 9 H), 1.31–1.15 (m, 2 H).

***tert*-Butyl 4-[({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)methyl]piperidine-1-carboxylate (38).** A mixture of 7-(3,5-dimethylisoxazol-4-yl)-8-hydroxy-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one **13** (63 mg, 0.152 mmol), *tert*-butyl 4-{[(methylsulfonyl)oxy]methyl}piperidine-1-carboxylate (59 mg, 0.201 mmol) and potassium carbonate (34 mg, 0.246 mmol) in DMF (1.5 mL) was stirred under nitrogen at 100 ºC and for 2.5 h. The reaction mixture was allowed to cool to rt, diluted with EtOAc (8 mL) and washed with brine (8 mL). The aqueous layer was separated and extracted with EtOAc (8 mL), the organic layers combined and passed through a hydrophobic frit. The solvent was removed by rotary evaporation and the residue dissolved in MeOH (1 mL). The solution was applied to a MeOH-preconditioned 2 g SCX-2 cartridge. The cartridge was washed with MeOH (10 mL) followed by 2 M ammonia in MeOH solution (10 mL). The basic wash was evaporated in vacuo and the material used directly in the next step.

7-(3,5-Dimethylisoxazol-4-yl)-3-methyl-8-(piperidin-4-ylmethoxy)-1-[1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one (39). Crude *tert*-Butyl 4-[({7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)methyl]piperidine-1-carboxylate **38** was dissolved in 1,4-dioxane (0.7 mL). To the solution was added 4 M HCl in 1,4-dioxane solution (0.5 mL, 0.152 mmol) and the mixture left to stir at rt for 1 h. The reaction was concentrated under a stream of nitrogen and the residue purified by MDAP (Method B). The appropriate fractions were combined and the solvent removed by rotary evaporation to give the title compound as a green solid (53 mg, 0.103 mmol, 68% yield).

^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.77 (s, 1 H), 8.58 (d, J = 4.8 Hz, 1 H), 7.77 (s, 1 H), 7.77–7.71 (m, 1 H), 7.41 (d, J = 7.8 Hz, 1 H), 7.31 (dd, J = 7.8, 4.8 Hz, 1 H), 6.97 (s, 1 H), 6.29 (q, J = 7.3 Hz, 1 H), 3.64–3.60 (m, 1 H), 3.59 (s, 3 H), 3.28 (dd, J = 9.4, 6.8 Hz, 1 H), 2.96–2.89 (m, 2 H), 2.46–2.41 (m, 1 H), 2.23 (s, 3 H), 2.05 (d, J = 7.3 Hz, 3 H), 2.03 (s, 3 H), 1.77–1.65 (m, 1 H), 1.57–1.47 (m, 2 H), 1.13–0.99 (m, 2 H), one piperidine proton signal obscured by DMSO, N*H* signal not resolved; % e.e. undetermined; LC-MS (Method B) $[M + H]^+$ = 513, R_t 0.78 min.

7-(3,5-Dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl trifluoromethanesulfonate (40). A solution of 7-(3,5-dimethylisoxazol-4-yl)-8-hydroxy-3-methyl-1-[(*R*)-1-(pyridin-2-yl)ethyl]-1*H*-imidazo[4,5-*c*]quinolin-2(3*H*)-one **13** (4.42g, 10.64 mmol) in DCM (20 mL) and pyridine (2.58 mL) under nitrogen was cooled in an ice bath and Tf_2O (2.16 ml, 12.77 mmol) added dropwise. The mixture was stirred for 2 h allowing to warm to rt. Further Tf_2O (0.05 mL) was added dropwise and the mixture stirred for 2 h. The reaction mixture was washed with water (2 × 20 mL), dried and evaporated. The residue was purified by column chromatography using a gradient of 0–10% 2 M ammonia in MeOH/DCM) to give the title compound as a beige foam (5.25 g, 9.59 mmol, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (s, 1 H), 8.64 (d, J = 4.8 Hz, 1 H), 8.03 (s, 1 H), 7.85 (br s, 1 H), 7.69 (td, J = 7.8, 1.8 Hz, 1 H), 7.42 (d, J = 7.8 Hz, 1 H), 7.30–7.23 (m, 1 H), 6.42 (q, J = 7.3 Hz, 1 H), 3.72 (s, 3 H), 2.33 (s, 3 H), 2.19 (s, 3 H), 2.10 (d, J = 7.3 Hz, 3 H); % e.e. undetermined; LC-MS (Method A) $[M + H]^+$ = 548, R_t 1.08 min.

Ethyl 7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinoline-8-carboxylate (41). A 100 mL 3-necked flask was charged with palladium diacetate (15 mg, 0.07 mmol), 1,3-bis(diphenylphosphino)propane (27 mg, 0.07 mmol), 7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-

imidazo[4,5-*c*]quinolin-8-yl trifluoromethanesulfonate **40** (500 mg, 0.91 mmol), anhydrous DMF (10 mL), EtOH (8 mL, 137 mmol) and triethylamine (0.28 mL, 2.01 mmol). The flask was fitted with a reflux condenser and the apparatus purged with CO gas. A balloon of CO gas was fitted to the top of the condenser and the mixture stirred at 70 ºC for 3 h. The reaction mixture was evaporated in vacuo and the resulting gum dissolved in EtOAc (10 mL). The organic layer was washed with 10% LiCl (aq) (10 mL) and then water (2 × 10 mL). The organic layer was passed through a hydrophobic frit and the solvent evaporated in vacuo. The crude material was purified on a 100 g silica cartridge using a gradient of 0–15% MeOH in DCM over 12 column volumes. The appropriate fractions were combined, and the solvent evaporated in vacuo to give the title compound as a light yellow foam (430 mg, 0.91 mmol, 100% yield). mp 90–92 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 1 H), 8.54 (d, J = 4.8 Hz, 1 H), 8.15 (br s, 1 H), 7.91 (s, 1 H), 7.78 (dd, J = 7.8, 7.3 Hz, 1 H), 7.47 (d, J = 7.8 Hz, 1 H), 7.31 (dd, J = 7.3, 4.8 Hz, 1 H), 6.33 (q, J = 7.3 Hz, 1 H), 4.19–4.05 (m, 2 H), 3.30 (s, 3 H), 2.20 (s, 3 H), 2.05 (d, J = 7.3 Hz, 3 H), 1.96 (s, 3 H), 1.18 (t, J = 7.1 Hz, 3 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.6, 163.3, 158.2, 158.5, 153.6, 149.2, 145.0, 137.3, 135.8, 133.7, 133.5, 129.0, 126.5, 126.3, 124.2, 122.6, 120.9, 115.2, 113.4, 60.9, 54.9, 27.8, 17.1, 13.8, 10.9, 10.0; % e.e. undetermined; LC-MS (Method A) $[M + H]^+$ = 472, R_t 0.87 min; HRMS $[M + H]^+$ calcd for $C_{26}H_{26}N_5O_4$ 472.1979, found 472.1972.

7-(3,5-Dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinoline-8-carboxylic acid (42). A solution of ethyl 7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[(*R*)-1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinoline-8-carboxylate **41** (300 mg, 0.64 mmol) in EtOH (2 mL) was treated with 2 M NaOH (aq) (0.33 mL, 0.66 mmol) and the reaction mixture stirred at rt for 15 h. Further 2 M NaOH (aq) (0.150 mL) was added and the mixture stirred at 50 ºC for 3 h. The reaction mixture was evaporated in vacuo and

the crude material purified by MDAP (Method A). The appropriate fractions were combined and the solvent removed by rotary evaporation to give the title compound (50 mol% formic acid) as an off-white solid (191 mg, 0.41 mmol, 64% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 353 K) δ 9.04 (s, 1 H), 8.52 (d, J = 4.8 Hz, 1 H), 8.30 (s, 1 H), 8.14 (s, 0.5 H, HCO$_2$H), 7.85 (s, 1 H), 7.74 (dd, J = 7.8, 7.3 Hz, 1 H), 7.44 (d, J = 7.8 Hz, 1 H), 7.28 (dd, J = 7.3, 4.8 Hz, 1 H), 6.33 (q, J = 7.3 Hz, 1 H), 3.63 (s, 3 H), 2.21 (s, 3 H), 2.07 (d, J = 7.3 Hz, 3 H), 1.99 (s, 3 H); % e.e. undetermined; LC-MS (Method A) $[M + H]^+$ = 444, R_t 0.61 min.

***tert*-Butyl 2-{7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}acetate (43).** A mixture of 7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl trifluoromethanesulfonate **40** (250 mg, 0.457 mmol) and $Pd(PPh_3)_4$ (53 mg, 0.046 mmol) in a sealed vessel under nitrogen was diluted with anhydrous NMP (0.5 mL) and [2-(*tert*-butoxy)-2-oxoethyl]zinc(II) chloride (0.5 M solution in THF) (2.5 mL, 1.250 mmol). The mixture was heated in a Biotage I60 microwave at 90 ºC for 20 min. The reaction mixture was diluted with sat. NH_4Cl (aq) (2 mL) and extracted with EtOAc (3 × 2 mL). The organic extracts were combined and passed through a hydrophobic frit. The solvent was removed in vacuo, the residue loaded in DCM (2 mL) and purified on a 100 g silica cartridge using a gradient of 0–100 % acetone in cyclohexane over 12 column volumes. The appropriate fractions were combined, the solvent removed by rotary evaporation and the solid purified by MDAP (Method B). The appropriate fractions were combined and the solvent removed by rotary evaporation to give the title compound as an off-white solid (39 mg, 0.076 mmol, 17% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.92 (s, 1 H), 8.56 (d, J = 4.8 Hz, 1 H), 7.81 (s, 1 H), 7.78 (s, 1 H), 7.77–7.72 (m, 1 H), 7.44 (d, J = 7.8 Hz, 1 H), 7.27 (dd, J = 7.8, 4.8 Hz, 1 H), 6.35 (q, J = 7.2 Hz, 1 H), 3.60 (s, 3 H), 3.36 (br s, 2 H), 2.20

(s, 3 H), 2.08 (d, J = 7.2 Hz, 3 H), 2.01 (s, 3 H), 1.32 (s, 9 H); % e.e. undetermined; LC-MS (Method A) $[M + H]^+$ = 514, R_t 0.91 min.

2-{7-(3,5-Dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}acetic acid (44). A solution of *tert*-butyl 2-{7-(3,5-dimethylisoxazol-4-yl)-3-methyl-2-oxo-1-[1-(pyridin-2-yl)ethyl]-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-8-yl}acetate **43** (35 mg, 0.068 mmol) in DCM (0.5 mL) was treated with TFA (0.5 mL, 6.49 mmol) and the mixture left to stand at rt in a stoppered vessel for 36 h. The reaction mixture was evaporated under a stream of nitrogen and was purified by MDAP (Method C). The appropriate fractions were combined and the solvent evaporated under a stream of nitrogen. The solid was dissolved in MeOH (0.5 mL) and applied to 0.5 g MeOH-preconditioned SCX-2 cartridge. The cartridge was washed with MeOH (3 mL) followed by 2 M ammonia in MeOH solution (3 mL). The basic wash was evaporated under a stream of nitrogen to give the title compound as an off white solid (11 mg, 0.024 mmol, 35% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 8.94–8.88 (m, 1 H), 8.56 (d, J = 4.8 Hz, 1 H), 7.81–7.77 (m, 2 H), 7.77–7.71 (m, 1 H), 7.44 (d, J = 8.1 Hz, 1 H), 7.27 (dd, J = 7.3, 4.8 Hz, 1 H), 6.35 (q, J = 7.2 Hz, 1 H), 3.60 (s, 3 H), 3.42–3.30 (m, 2 H), 2.19 (s, 3 H), 2.08 (d, J = 7.2 Hz, 3 H), 2.00 (s, 3 H); % e.e. undetermined; LC-MS (Method A) $[M + H]^+$ = 458, R_t 0.59 min.

1-(Benzyloxy)-2-bromo-4-nitrobenzene (47). To a slurry of NaH (60% dispersion in mineral oil) (2.8 g, 70.0 mmol) and DMA (60 mL) was added benzyl alcohol (4.75 mL, 45.7 mmol) dropwise with stirring under nitrogen. The mixture was stirred for 2 h at rt and then cooled to 0 °C. 2-Bromo-1-fluoro-4-nitrobenzene **46** (11 g, 50.0 mmol) was added to the cooled solution and the mixture stirred for 1 h. The reaction mixture was poured onto sat. NH_4Cl (aq) (80 mL), stirred for 10 min, filtered and washed with water. The solid was triturated with diethyl ether (25 mL) and

filtered to give the title compound as a tan solid (11.25 g, 36.5 mmol, 73% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.45 (d, J = 2.8 Hz, 1 H), 8.28 (dd, J = 9.1, 2.8 Hz, 1 H), 7.53–7.47 (m, 2 H), 7.47–7.40 (m, 3 H), 7.40–7.34 (m, 1 H), 5.39 (s, 2 H); LC-MS (Method A) no mass ion detected, R_t 1.30 min.

4-[2-(Benzyloxy)-5-nitrophenyl]-3,5-dimethylisoxazole (48). Nitrogen was bubbled through a mixture of 1-(benzyloxy)-2-bromo-4-nitrobenzene **47** (10.73 g, 34.8 mmol), cesium carbonate (23 g, 70.6 mmol), DME (100 mL) and water (40 mL) for 30 min. (3,5-Dimethylisoxazol-4-yl)boronic acid (9.75 g, 69.2 mmol) and PEPPSI-IPr catalyst (0.6 g, 0.883 mmol) were added and the mixture stirred at 90 °C for 5 h under nitrogen. The reaction was allowed to cool to rt then EtOAc (150 mL) and water (75 mL) added. The layers were separated and the organic layer washed with 10% Na_2SO_3 (aq) (75 mL) followed by brine (75 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The solid was triturated with diethyl ether (50 mL), filtered and washed with cyclohexane (100 mL). The solid was dried in a vacuum oven to give the title compound as a light brown solid (9.72 g, 30.0 mmol, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (dd, J = 9.1, 2.8 Hz, 1 H), 8.07 (d, J = 2.8 Hz, 1 H), 7.41–7.31 (m, 3 H), 7.30–7.25 (m, 2 H), 7.13 (d, J = 9.1 Hz, 1 H), 5.19 (s, 2 H), 2.30 (s, 3 H), 2.16 (s, 3 H); LC-MS (Method A) $[M + H]^+$ = 325, R_t 1.19 min.

4-(Benzyloxy)-3-(3,5-dimethylisoxazol-4-yl)aniline (49). A mixture of 4-[2-(benzyloxy)-5-nitrophenyl]-3,5-dimethylisoxazole **48** (9.2 g, 28.4 mmol), iron powder (6.0 g, 107 mmol) and ammonium chloride (12 g, 224 mmol) was suspended in EtOH (400 mL) and water (100 mL) and stirred at rt for 15 h. The reaction mixture was filtered through a pad of Celite and the solid washed with EtOH. The filtrate was evaporated under vacuum and the residue partitioned between EtOAc (150 mL) and water (150 mL). The organic layer was separated, washed with further water (100

mL) and dried over $MgSO_4$. The solvent was removed under vacuum to give the title compound as a dark brown oil (8.2 g, 27.9 mmol, 98% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.25 (m, 3 H), 7.25–7.20 (m, 2 H), 6.91 (d, J = 8.6 Hz, 1 H), 6.69 (dd, J = 8.6, 2.9 Hz, 1 H), 6.51 (d, J = 2.9 Hz, 1 H), 4.92 (s, 2 H), 3.53 (br s, 2 H), 2.29 (s, 3 H), 2.18 (s, 3 H); LC-MS (Method A) $[M + H]^+$ = 295, R_t 0.75 min, 85% purity by LC-MS UV (10% starting material remains).

Diethyl 2-({[4-(benzyloxy)-3-(3,5-dimethylisoxazol-4-yl)phenyl]amino}methylene)malonate (50). A mixture of 4-(benzyloxy)-3-(3,5-dimethylisoxazol-4-yl)aniline **49** (8.2 g, 27.9 mmol) and diethyl 2-(ethoxymethylene)malonate (5.6 mL, 28.0 mmol) was stirred at 130 ºC for 1 h. The brown oil was concentrated under reduced pressure. The residue was loaded in DCM (20 mL) and purified on a 330 g silica cartridge using a gradient of 0–100% EtOAc in DCM over 10 column volumes. The appropriate fractions were combined and the solvent removed by rotary evaporation. The resulting solid was triturated with cyclohexane, filtered and dried in a vacuum oven to give the title compound as a light brown solid (9.6 g, 20.67 mmol, 74% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.02 (d, J = 13.5 Hz, 1 H), 8.44 (d, J = 13.5 Hz, 1 H), 7.40–7.24 (m, 5 H), 7.15 (dd, J = 8.8, 2.9 Hz, 1 H), 7.07 (d, J = 8.8 Hz, 1 H), 6.94 (d, J = 2.9 Hz, 1 H), 5.06 (s, 2 H), 4.32 (q, J = 7.1 Hz, 2 H), 4.26 (q, J = 7.1 Hz, 2 H), 2.31 (s, 3 H), 2.18 (s, 3 H), 1.40 (t, J = 7.1 Hz, 3 H), 1.34 (t, J = 7.1 Hz, 3 H); LC-MS (Method A) $[M + H]^+$ = 465, R_t 1.30 min.

Ethyl 6-(benzyloxy)-7-(3,5-dimethylisoxazol-4-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (51). Diphenyl ether (75 mL) was heated with stirring to 260 ºC (internal). Solid diethyl 2-({[4-(benzyloxy)-3-(3,5-dimethylisoxazol-4-yl)phenyl]amino}methylene)malonate **50** (9.6 g, 20.67 mmol) was added in portions, followed by diphenyl ether (10 mL). The mixture was stirred for 20 min. The mixture was allowed to cool to rt and diluted with DCM (20 mL). The

solution was loaded onto a 330 g silica cartridge and purified using a gradient of 0–20% MeOH in DCM over 10 column volumes. The appropriate fractions were combined and the solvent removed by rotary evaporation to give the title compound as a light brown solid (7.8 g, 18.64 mmol, 90% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.27 (br s, 1 H), 8.55 (s, 1 H), 7.82 (s, 1 H), 7.53 (s, 1 H), 7.40–7.29 (m, 5 H), 5.24 (s, 2 H), 4.22 (q, J = 7.1 Hz, 2 H), 2.30 (s, 3 H), 2.10 (s, 3 H), 1.29 (t, J = 7.1 Hz, 3 H); LC-MS (Method A) $[M + H]^+$ = 419, R_t 0.93 min, 85% purity by LC-MS UV (9% decarboxylated material present).

6-(Benzyloxy)-7-(3,5-dimethylisoxazol-4-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (52). To ethyl 6-(benzyloxy)-7-(3,5-dimethylisoxazol-4-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **51** (7.8 g, 18.64 mmol) was added EtOH (50 mL) and 2 M NaOH (aq) (30 mL, 60.0 mmol) and the mixture stirred at 95 ºC for 3 h. The reaction was allowed to cool to rt and the volatiles evaporated under vacuum. The remaining mixture was acidified to pH = 1 with 12.5% HCl (aq) (~30 mL) and the resulting precipitate isolated by vacuum filtration. The solid was washed with water (200 mL) and diethylether (50 mL), and then dried in a vacuum oven overnight to give the title compound as an off-white solid (7.2 g, 18.44 mmol, 99% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 15.64 (br s, 1 H), 8.85 (s, 1 H), 7.90 (s, 1 H), 7.76 (s, 1 H), 7.43–7.30 (m, 5 H), 5.31 (s, 2 H), 2.31 (s, 3 H), 2.12 (s, 3 H); LC-MS (Method A) $[M + H]^+$ = 391, R_t 1.00 min, 82% purity by LC-MS UV (10% decarboxylated material present).

6-(Benzyloxy)-7-(3,5-dimethylisoxazol-4-yl)quinolin-4(1*H*)-one (53). Diphenyl ether (70 mL) was heated with stirring to 260 ºC (internal). Solid 6-(benzyloxy)-7-(3,5-dimethylisoxazol-4-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **52** (7.2 g, 18.44 mmol) was added in one portion followed by diphenyl ether (10 mL). The mixture was stirred for 20 min. The reaction was allowed to cool to rt and the solution applied to a 330 g silica cartridge with DCM (20 mL). The mixture

was purified using a gradient of 0–20% MeOH in DCM over 10 column volumes. The appropriate fractions were combined and the solvent removed by rotary evaporation to give the title compound as a brown solid (5.7 g, 16.46 mmol, 89% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.72 (br s, 1 H), 7.92–7.86 (m, 1 H), 7.75 (s, 1 H), 7.44 (s, 1 H), 7.41–7.29 (m, 5 H), 6.03 (d, J = 7.2 Hz, 1 H), 5.21 (s, 2 H), 2.29 (s, 3 H), 2.11 (s, 3 H); LC-MS (Method A) $[M + H]^+$ = 347, R_t 0.83 min.

6-(Benzyloxy)-7-(3,5-dimethylisoxazol-4-yl)-3-nitroquinolin-4(1*H*)-one (54). To a 500 mL round bottomed flask containing 6-(benzyloxy)-7-(3,5-dimethylisoxazol-4-yl)quinolin-4(1*H*)-one **53** (5.7 g, 16.46 mmol) was added propionic acid (75 mL, 1002 mmol) followed by 70% nitric acid (1.6 mL, 35.8 mmol). A suspension formed which was stirred at 100 ºC for 1.5 h. The reaction mixture was allowed to cool to rt, filtered under vacuum and washed with cyclohexane (100 mL). The solid was dried in a vacuum oven to give the title compound as a tan solid (3.7 g, 9.45 mmol, 57% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.97 (br s, 1 H), 9.18 (s, 1 H), 7.91 (s, 1 H), 7.63 (s, 1 H), 7.42–7.29 (m, 5 H), 5.29 (s, 2 H), 2.30 (s, 3 H), 2.11 (s, 3 H); LC-MS (Method A) $[M + H]^+$ = 392, R_t 0.94 min.

4-[6-(Benzyloxy)-4-chloro-3-nitroquinolin-7-yl]-3,5-dimethylisoxazole (55). To 6-(benzyloxy)-7-(3,5-dimethylisoxazol-4-yl)-3-nitroquinolin-4(1*H*)-one **54** (3.53 g, 9.02 mmol) was added $POCl_3$ (20 mL, 215 mmol) and the suspension stirred at 100 ºC under nitrogen for 1 h. The reaction mixture was allowed to cool to rt and evaporated under vacuum. Toluene (20 mL) was added and the mixture evaporated. The residue was dissolved in DCM (25 mL) and sat. $NaHCO_3$ (aq) (25 mL) was added. The mixture was stirred vigorously for 30 min and the organic layer separated. The aqueous layer was extracted with DCM (25 mL), the organics combined and passed through a hydrophobic frit. The solvent was removed under vacuum and the resulting solid triturated with cyclohexane:diethylether (8:2) (40 mL). The solid was dried in vacuo to give the

title compound as a light brown solid (3.54 g, 8.64 mmol, 96% yield). mp 230–232 ºC; IR (solid) ν (cm^{-1}) 3130, 1619, 1556, 1526, 1420, 1381, 1331, 1227, 1023, 739; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.16 (s, 1 H), 8.01 (s, 1 H), 7.82 (s, 1 H), 7.47–7.33 (m, 5 H), 5.32 (s, 2 H), 2.37 (s, 3 H), 2.23 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.1, 159.3, 157.3, 144.9, 142.6, 141.7, 135.0, 134.2, 133.0, 129.2, 128.9 (2 C), 128.7, 127.4 (2 C), 126.7, 111.7, 104.7, 71.3, 12.0, 10.9; LC-MS (Method A) $[M + H]^+$ = 410, 412, R_t 1.32 min; HRMS $[M + H]^+$ calcd for $C_{21}H_{17}{}^{35}ClN_3O_4$ 410.0902, found 410.0897.

6-(Benzyloxy)-7-(3,5-dimethylisoxazol-4-yl)-3-nitro-*N*-[(*R*)-1-phenylethyl]quinolin-4-amine (56). A solution of 4-[6-(benzyloxy)-4-chloro-3-nitroquinolin-7-yl]-3,5-dimethylisoxazole **55** (5 g, 12.20 mmol) in anhydrous NMP (25 mL) was treated with ^{i}Pr$_2$NEt (4.26 mL, 24.40 mmol) followed by (*R*)-1-phenylethanamine (1.71 mL, 13.42 mmol). The solution was stirred under nitrogen at rt for 2.5 h. The reaction mixture was diluted with water (50 mL) and extracted with EtOAc (75 mL). The organic layer was washed with 10% LiCl (aq) (2 × 50 mL) followed by water (50 mL) and passed through a hydrophobic frit. The solvent was removed in vacuo and dried in a vacuum oven to give the title compound as a yellow foam (5.96 g, 12.05 mmol, 99% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.54 (d, J = 7.8 Hz, 1 H), 9.27 (s, 1 H), 7.70 (s, 1 H), 7.53–7.46 (m, 2 H), 7.44–7.37 (m, 3 H), 7.36–7.29 (m, 1 H), 7.28–7.21 (m, 3 H), 7.00–6.95 (m, 2 H), 5.32 (dq, J = 7.8, 6.7 Hz, 1 H), 4.46 (d, J = 11.8 Hz, 1 H), 4.07 (d, J = 11.8 Hz, 1 H), 2.20 (s, 3 H), 2.07 (s, 3 H), 1.69 (d, J = 6.7 Hz, 3 H); LC-MS (Method A) $[M + H]^+$ = 495, R_t 1.31 min.

6-(Benzyloxy)-7-(3,5-dimethylisoxazol-4-yl)-N^4-[(*R*)-1-phenylethyl]quinoline-3,4-diamine (57). Iron powder (5.36 g, 96 mmol) was added to a solution of 6-(benzyloxy)-7-(3,5-dimethylisoxazol-4-yl)-3-nitro-*N*-[(*R*)-1-phenylethyl]quinolin-4-amine **56** (5.93 g, 11.99 mmol) in acetic acid (50 mL). The mixture was stirred in an open vessel at rt for 1 h. EtOAc (50 mL) was

added to the flask and the mixture stirred for 1 h. The mixture was filtered through a Celite cartridge which was washed with further EtOAc (100 mL). The filtrate was washed with 0.5 M NaOH (aq) (2 × 100 mL) and water (100 mL). The organic layer was separated, passed through a hydrophobic frit and the solvent removed by rotary evaporation. The resulting foam was dissolved in MeOH (10 mL) and applied to a MeOH-preconditioned 50 g aminopropyl cartridge. The cartridge was washed with MeOH (160 mL) and the solvent evaporated under vacuum to give the title compound as a light brown foam (4.62 g, 9.94 mmol, 83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (s, 1 H), 7.73 (s, 1 H), 7.41–7.25 (m, 10 H), 6.99 (s, 1 H), 5.02–4.93 (m, 2 H), 4.52 (q, J = 6.7 Hz, 1 H), 3.69 (br s, 2 H), 3.56 (br s, 1 H), 2.34 (s, 3 H), 2.21 (s, 3 H), 1.61 (d, J = 6.7 Hz, 3 H); LC-MS (Method A) $[M + H]^+$ = 465, R_t 0.99 min.

***N*-[6-(Benzyloxy)-7-(3,5-dimethylisoxazol-4-yl)-4-{[(*R*)-1-phenylethyl]amino}quinolin-3-yl]-2-methoxyacetamide (58).** A solution of 6-(benzyloxy)-7-(3,5-dimethylisoxazol-4-yl)-N^4-[(*R*)-1-phenylethyl]quinoline-3,4-diamine **57** (1.89 g, 4.07 mmol) and pyridine (0.50 mL, 6.18 mmol) in DCM (20 mL) was cooled to 0 ºC and 2-methoxyacetyl chloride (0.41 mL, 4.48 mmol) added. The mixture was stirred under nitrogen at 0 ºC for 10 min and allowed to warm to rt over 3.5 h. The reaction mixture was washed sequentially with 0.5 M HCl (aq) (20 mL) and water (2 × 20 mL). The organic layer was separated, passed through a hydrophobic frit and the solvent removed by rotary evaporation to give the title compound as a light brown foam (2.14 g, 3.99 mmol, 98% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (s, 1 H), 8.25 (s, 1 H), 7.82 (s, 1 H), 7.40–7.29 (m, 8 H), 7.26–7.21 (m, 3 H), 4.92–4.85 (m, 2 H), 4.62 (dq, J = 8.6, 6.8 Hz, 1 H), 4.27 (d, J = 8.6 Hz, 1 H), 4.10 (s, 2 H), 3.53 (s, 3 H), 2.34 (s, 3 H), 2.21 (s, 3 H), 1.61 (d, J = 6.8 Hz, 3 H); LC-MS (Method A) $[M + H]^+$ = 537, R_t 0.91 min.

4-{8-(Benzyloxy)-2-(methoxymethyl)-1-[(*R*)-1-phenylethyl]-1*H*-imidazo[4,5-*c*]quinolin-7-yl}-3,5-dimethylisoxazole (59). A solution of *N*-[6-(benzyloxy)-7-(3,5-dimethylisoxazol-4-yl)-4-{[(*R*)-1-phenylethyl]amino}quinolin-3-yl]-2-methoxyacetamide **58** (2.14 g, 3.99 mmol) in propionic acid (6.0 mL, 80 mmol) was stirred at 140 ºC under nitrogen for 2 h. The reaction mixture was allowed to cool to rt and the solvent removed in vacuo. The residue was dissolved in DCM (25 mL) and washed with 1 M NaOH (aq) (25 mL). The aqueous layer was extracted with DCM (25 mL), the organic layers combined and passed through a hydrophobic frit. The solvent was removed by rotary evaporation, the foam dissolved in DCM (5 mL) and purified on a 100 g silica cartridge using a gradient of 0–15% MeOH in DCM over 12 column volumes. The appropriate fractions were combined and the solvent was removed by rotary evaporation to give the title compound as a light brown foam (1.87 g, 3.61 mmol, 90% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 9.15 (s, 1 H), 7.96 (s, 1 H), 7.46–7.30 (m, 8 H), 7.24–7.20 (m, 2 H), 7.09 (s, 1 H), 6.47 (q, J = 7.1 Hz, 1 H), 4.88–4.85 (m, 2 H), 4.80 (d, J = 11.9 Hz, 1 H), 4.41 (d, J = 11.9 Hz, 1 H), 3.40 (s, 3 H), 2.24 (s, 3 H), 2.07–2.03 (m, 6 H); LC-MS (Method A) $[M + H]^+$ = 519, R_t 1.08 min.

7-(3,5-Dimethylisoxazol-4-yl)-2-(methoxymethyl)-1-[(*R*)-1-phenylethyl]-1*H*-imidazo[4,5-*c*]quinolin-8-ol (60). A solution of 4-{8-(benzyloxy)-2-(methoxymethyl)-1-[(*R*)-1-phenylethyl]-1*H*-imidazo[4,5-*c*]quinolin-7-yl}-3,5-dimethylisoxazole **59** (1.81 g, 3.49 mmol) in EtOH (20 mL) was added to 5% palladium on carbon (180 mg, 1.692 mmol) and stirred at rt under atmospheric hydrogen for 15 h. The reaction mixture was filtered through Celite and the cake washed with EtOH (50 mL). The filtrate was evaporated under vacuum and the residue dissolved in EtOH (40 mL). The solution was added to 5% palladium on carbon (360 mg, 3.38 mmol) and stirred at rt under atmospheric hydrogen for 20 h. The reaction mixture was filtered through Celite and the cake washed with EtOH (50 mL). The filtrate was evaporated under vacuum and the residue

dissolved in DCM (8 mL). The solution was loaded onto a 220 g silica cartridge and purified using a gradient of 0–15% MeOH in DCM over 10 column volumes. The appropriate fractions were combined and the solvent removed by rotary evaporation to give an off-white solid which was purified by MDAP (Method B). The appropriate fractions were combined and the solvent removed by rotary evaporation to give the title compound as a light-yellow solid (923 mg, 2.154 mmol, 62% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 9.50 (br s, 1 H), 9.04 (s, 1 H), 7.89 (s, 1 H), 7.45–7.23 (m, 6 H), 6.51 (q, J = 7.1 Hz, 1 H), 4.69 (d, J = 12.9 Hz, 1 H), 4.62 (d, J = 12.9 Hz, 1 H), 3.33 (s, 3 H), 2.31 (s, 3 H), 2.17 (d, J = 7.1 Hz, 3 H), 2.14 (s, 3 H); LC-MS (Method A) $[M + H]^+$ = 429, R_t 0.72 min.

Fractions containing a minor peak were combined and the solvent removed by rotary evaporation to give byproduct (*R*)-1-{2-(methoxymethyl)-8-methyl-1-(1-phenylethyl)-1*H*-furo[2,3-*g*]imidazo[4,5-*c*]quinolin-7-yl}ethanone **63** as a light-yellow foam (129 mg, 0.312 mmol, 9% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 9.24 (s, 1 H), 8.71 (s, 1 H), 7.70 (s, 1 H), 7.44–7.36 (m, 2 H), 7.36–7.25 (m, 3 H), 6.57 (q, J = 7.1 Hz, 1 H), 4.82 (s, 2 H), 3.38 (s, 3 H), 2.79 (s, 3 H), 2.67 (s, 3 H), 2.13 (d, J = 7.1 Hz, 3 H); LC-MS (Method A) $[M + H]^+$ = 414, R_t 0.81 min.

(3*S*)-*tert*-Butyl 3-[({7-(3,5-dimethylisoxazol-4-yl)-2-(methoxymethyl)-1-[(*R*)-1-phenylethyl]-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)methyl]pyrrolidine-1-carboxylate (61). A mixture of 7-(3,5-dimethylisoxazol-4-yl)-2-(methoxymethyl)-1-[(*R*)-1-phenylethyl]-1*H*-imidazo[4,5-*c*]quinolin-8-ol **60** (233 mg, 0.544 mmol), potassium carbonate (107 mg, 0.778 mmol) and (*S*)-*tert*-butyl 3-{[(methylsulfonyl)oxy]methyl}pyrrolidine-1-carboxylate (190 mg, 0.680 mmol) in anhydrous DMF (1.2 mL) was stirred at 100 ºC under nitrogen for 3 h. The reaction mixture was allowed to cool to rt, diluted with 10% LiCl (aq) (10 mL) and extracted with EtOAc (3 × 10 mL). The organic layers were combined and passed through a hydrophobic frit. The solvent

was removed by rotary evaporation and the residue purified by MDAP (Method B). The appropriate fractions were combined and evaporated in vacuo to give the title compound as an off-white solid (191 mg, 0.312 mmol, 57% yield). ^{1}H NMR (400 MHz, DMSO-d_6, 373 K) δ 9.15 (s, 1 H), 7.93 (s, 1 H), 7.44–7.38 (m, 2 H), 7.37–7.31 (m, 3 H), 6.92 (s, 1 H), 6.49 (q, J = 7.0 Hz, 1 H), 4.93 (d, J = 13.0 Hz, 1 H), 4.88 (d, J = 13.0 Hz, 1 H), 3.71 (dd, J = 9.4, 6.4 Hz, 1 H), 3.41 (s, 3 H), 3.39–3.25 (m, 3 H), 3.13–3.07 (m, 1 H), 2.93–2.91 (m, 1 H), 2.49–2.41 (m, 1 H), 2.27 (s, 3 H), 2.11 (d, J = 7.0 Hz, 3 H), 2.08 (s, 3 H), 2.00–1.89 (m, 1 H), 1.65–1.54 (m, 1 H), 1.46 (s, 9 H); LC-MS (Method A) $[M + H]^+$ = 612, R_t 1.08 min; HRMS $[M + H]^+$ calcd for $C_{35}H_{42}N_5O_5$ 612.3181, found 612.3170.

4-{2-(Methoxymethyl)-1-[(*R*)-1-phenylethyl]-8-[(*S*)-pyrrolidin-3-ylmethoxy]-1*H*-imidazo[4,5-*c*]quinolin-7-yl}-3,5-dimethylisoxazole (62). A solution of (3*S*)-*tert*-Butyl 3-[({7-(3,5-dimethylisoxazol-4-yl)-2-(methoxymethyl)-1-[(*R*)-1-phenylethyl]-1*H*-imidazo[4,5-*c*]quinolin-8-yl}oxy)methyl]pyrrolidine-1-carboxylate **61** (186 mg, 0.304 mmol) in anhydrous 1,4-dioxane (2 mL) was treated with 4 M HCl in 1,4-dioxane (2 mL, 8.00 mmol) and the mixture allowed to stand in a stoppered vessel for 1 h. The reaction mixture was evaporated in vacuo and the solid dissolved in MeOH (2 mL). The solution was applied to a MeOH-preconditioned 5 g SCX-2 cartridge which was then washed with MeOH (30 mL) followed by 2 M ammonia in MeOH solution (30 mL). The basic wash was evaporated in vacuo to give the title compound as an off-white solid (150 mg, 0.293 mmol, 96% yield). mp 130–132 ºC; $[\alpha]_D^{21}$ −27 (*c* 0.5, $CHCl_3$); IR (solid) ν (cm^{-1}) 2929, 1618, 1495, 1444, 1393, 1367, 1215, 1095; ^{1}H NMR (400 MHz, DMSO-d_6, 393 K) δ 9.13 (s, 1 H), 7.92 (s, 1 H), 7.44–7.37 (m, 2 H), 7.37–7.29 (m, 3 H), 6.95 (s, 1 H), 6.48 (q, J = 7.0 Hz, 1 H), 4.91 (d, J = 13.0 Hz, 1 H), 4.87 (d, J = 13.0 Hz, 1 H), 3.65 (dd, J = 9.3, 6.3 Hz, 1 H), 3.41 (s, 3 H), 3.19–3.12 (m, 1 H), 2.92–2.81 (m, 3 H), 2.44 (dd, J = 10.6, 6.0 Hz, 1 H),

2.34–2.22 (m, 4 H), 2.11 (d, J = 7.0 Hz, 3 H), 2.07 (s, 3 H), 1.85–1.74 (m, 1 H), 1.40–1.29 (m, 1 H), N*H* not resolved; ^{13}C NMR (100 MHz, DMSO-d_6, 393 K) δ 165.2, 158.3, 153.9, 151.8, 142.7, 139.7, 139.6, 137.1, 132.5, 132.4, 128.6 (2C), 127.0, 125.1 (2C), 120.2, 117.4, 111.8, 104.1, 71.3, 66.8, 57.5, 54.1, 49.4, 45.6, 38.1, 28.5, 18.7, 10.6, 9.5; >99% d.e. determined by HPLC analysis on a Chiralpak AD column (250 × 4.6 mm, 10 μm), elution with 20% EtOH in heptane containing 0.1% isopropylamine, flow rate 1 mL/min, UV detection at 235 nm; LC-MS (Method A) $[M + H]^+$ = 512, R_t 0.59 min; HRMS $[M + H]^+$ calcd for $C_{30}H_{34}N_5O_3$ 512.2656, found 512.2650.

Mutant TR-FRET assays. Compounds were screened against *N*-terminal 6His-tagged single mutant tandem bromodomain proteins in a dose-response format using an Alexa Fluor 647 derivative of I-BET762 (**63**, Supporting Information Method S2ii). Compounds were titrated from 10 mM in 100% DMSO and 100 nL transferred to a low volume black 384 well micro titre plate using a Labcyte Echo 555. A Thermo Scientific Multidrop Micro were used to dispense 5 μL of 10 nsM protein in 50 mM HEPES, 50 mM NaCl and 1 mM CHAPS, pH 7.4, in the presence of 50 nM compound **63** (~K_d concentration for the interaction between all BET single mutant tandem bromodomain proteins and **63**, except for BRDT Y66A, which used 200 nM). After equilibrating for 1 h in the dark at rt, the bromodomain protein:fluorescent ligand interaction was detected using TR-FRET following a 5 μL addition of 1.5 nM europium chelate labelled anti-6His antibody (Perkin Elmer, W1024, AD0111) in assay buffer. Time resolved fluorescence (TRF) was then detected on a TRF laser equipped Perkin Elmer Envision multimode plate reader (excitation = 337 nm; emission 1 = 615 nm; emission 2 = 665 nm; dual wavelength bias dichroic = 400 nm, 630 nm). TR-FRET ratio was calculated using the following equation: Ratio = ((Acceptor fluorescence at 665 nm) / (Donor fluorescence at 615 nm)) * 1000. TR-FRET ratio data was normalized to a mean of 16 replicates per micro titre plate of both 10 μM I-BET151 and 1% DMSO controls and

IC_{50} values determined for each of the compounds tested by fitting the fluorescence ratio data to a four parameter model: y = a + ((b – a) / (1 + (10 ^ x / 10 ^ c) ^ d) where 'a' is the minimum, 'b' is the Hill slope, 'c' is the IC_{50} and 'd' is the maximum. All compounds were screened against BRD4 (1–477) Y97A and Y390A with selected compounds also screened against BRD2 (1–473) Y113A and Y386A, BRD3 (1–435) Y73A and Y348A, and BRDT (1–397) Y66A and Y309A.

Mouse Pharmacokinetic (PK) studies. *Experimental protocol.* All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GlaxoSmithKline (GSK) Policy on the Care, Welfare and Treatment of Laboratory Animals. Mice were individually housed in a plastic solid bottom cage and had free access to food (5LF2 EURodent Diet 14% supplied by PMI Labdiet, Richmond, Indiana, USA) and water. There were no known contaminants in the diet or water at concentrations that could interfere with the outcome of this study. Temperature and humidity were nominally maintained at 21 °C ± 2 °C and 55% ± 10%, respectively. Three male CD1 mice (30–33 g, supplied by Charles River UK Ltd.) each received an intraperitoneal administration of **62** formulated in 10% Kleptose™ in saline at a concentration of 2 mg/mL and administered at 5 mL/kg to achieve a target dose of 10 mg/kg. The pH of the dose formulation was adjusted to 5 using 2 M HCl (aq). Following dose administration, serial blood samples (*ca*. 20 μL) were collected up to 12 h after the start of dosing, via direct puncture of the tail vein, a terminal sample was taken at 24 h post dose via cardiac puncture under terminal isoflurane anesthesia. Blood samples were collected into blank tubes, diluted with an equal volume of purified water and stored at −20 °C prior to analysis by LC-MS/MS.

Blood sample analysis. Diluted blood samples were extracted using protein precipitation with 250 μL of acetonitrile containing an analytical internal standard. An aliquot of the supernatant was

analyzed by reverse phase LC-MS/MS in positive ion mode. Samples were assayed against calibration standards prepared in control blood.

PK data analysis. PK profiles were obtained from the blood concentration-time profiles using non-compartmental analysis with WinNonlin Phoenix 6.3 (Certara, Princetown, NJ.)

Fraction unbound in blood. Control blood from CD1 mice was obtained on the day of experimentation from in house GSK stock animals. Control human blood was obtained on the day of experimentation from a single non-medicated consenting donor from in house GSK blood donation unit. The human biological samples were sourced ethically and their research use was in accordance with the terms of the informed consent. The fraction unbound was determined using rapid equilibrium dialysis technology (RED^{TM} plate [Linden Bioscience, Woburn, MA]) at concentrations of 200 ng/mL and 1000 ng/mL. Blood was dialyzed against phosphate buffered saline solution incubating the dialysis units at 37 °C for 4 h. Following incubation, aliquots of blood and buffer were matrix matched prior to analysis by LC-MS/MS. The unbound fraction was determined using the peak area ratios in buffer and in blood.

*Prediction of the in vivo response (target engagement) for compound **62** in the male CD1 mouse.*

Mean (n = 3 mice) blood concentration-time profiles adjusted for the free fraction in mouse blood (0.08) were transformed using an inhibitory sigmoid I_{max} model incorporating *in vitro* efficacy data to simulate response-time (target engagement) profiles for compound **62** following ip administration at 10 mg/kg.

The inhibitory sigmoid I_{max} model was defined as

$$Response = \frac{I_{max} * C^{\gamma}}{IC_{50}{}^{\gamma} + C^{\gamma}}$$

Where I_{max} is the maximum induced drug effect, IC_{50} is the unbound drug concentration at 50% of maximal effect, C is the blood concentration and γ is the sigmoidicity factor or hill slope.

The IC_{50} and hill slope were determined from *in vitro* studies investigating the inhibition of IL-6 production in LPS-stimulated human whole blood or from BRD4 BD1 and BD2 mutant TR-FRET assays. The IC_{50} determined in human whole blood was adjusted for the free fraction (0.17) and it was assumed that potency in human blood reflects potency in mouse blood. The TR-FRET assays were assumed to be protein free and no adjustment for free fraction was made.

ASSOCIATED CONTENT

Supporting Information

Protein production, determination of binding constants of fluorescent ligand **63** in wild type and mutant tandem BET proteins, NMR spectra, synthesis and characterization of compound **45**, LC-MS traces of compound **62**, diastereomeric purity of **62** by chiral HPLC, BET and BRD4 mutant TR-FRET data tables for tested compounds, magnified view of the crystal structure of **62** in BRD4 BD1 displaying distances, angles and B-factors for interactions of the pyrrolidine group, SPR experimental and sensorgrams, bromodomain binding assay protocol, data and concentration-response curves, nanoBRET method and concentration-response curves for compound **62**, protocols for phenotypic assays, developability and cross screening panel data for compound **62**. (PDF). Molecular formula strings and biological data for final compounds (CSV). This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

The atomic coordinates of **62** bound to BRD4 BD1 and BRD2 BD2 have been deposited with the Protein Data Bank under the accession codes 6swn and 6swo, respectively.[25]

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Author Contributions

The manuscript was written with contributions from all authors. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

AML, acute myeloid leukemia; ApoA1, apolipoprotein A1; BCP, bromodomain-containing protein; BET, bromodomain and extraterminal domain; BRD2/3/4, bromodomain-containing protein 2/3/4; CREBBP, cyclic adenosine monophosphate response element binding protein

binding protein; IL-6, interleukin 6; ITC, isothermal titration calorimetry; KAc, acetyl-lysine; MCP-1, monocyte chemotactic protein-1; MDAP, mass-directed autopreparative HPLC; MLL, mixed lineage leukemia; NMC, NUT midline carcinoma; NUT, nuclear protein in testis; PAFc, polymerase-associated factor complex; PEPPSI, pyridine enhanced precatalyst preparation stabilization and initiation; PBMC, peripheral blood mononuclear cell; Pol II, RNA polymerase II; P-TEFb, positive transcription elongation factor b; PTM, post-translational modification; qPCR, quantitative real-time polymerase chain reaction; SEC, superelongation complex; SPR, surface plasmon resonance; TNFα, tumor necrosis factor α; TR-FRET, time-resolved Förster (fluorescence) resonance energy transfer

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