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The design and synthesis of 5- and 6-isoxazolylbenzimidazoles as selective inhibitors of the BET bromodomains†‡

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Simple 1-substituted 5- and 6-isoxazolyl-benzimidazoles have been shown to be potent inhibitors of the BET bromodomains with selectivity over the related bromodomain of CBP. The reported inhibitors were prepared from simple starting materials in two steps followed by separation of the regioisomers or regioselectively in three steps.

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Bromodomains are discrete protein domains that selectively recognize acetyl lysine in proteins.[1] There are 61 bromodomains in proteins that have a variety of functions including histone acetyl transferases such as CBP (cyclic AMP response element-binding protein, binding protein), methyl transferases, transcriptional regulators such as BRD4 (bromodomain-containing protein 4) and chromatin remodelling complexes.[2] The BET family of bromodomain containing proteins is comprised of BRDT, BRD2, BRD3 and BRD4 each of which has two bromodomains that bind to acetylated histone tails.[3] Recently BET inhibitors have been shown to have potential for use in inflammatory disease, atherosclerosis, NUT midline carcinoma, acute leukaemia and lymphoma.[4–9]

Triazoloazepines such as (+)-JQ1 **1**, iBET762 (structure not shown) and isoxazoles such as compound **2** have been identified as potent BET inhibitors (Fig. 1).[4,9,10] Since then, a number of other templates incorporating the privileged isoxazole moiety such as in compounds **3** and **4** have been identified by researchers in the EpiNova group at GlaxoSmithKline.[6,11,12] As most known BET inhibitors are complex stereogenic molecules it would be advantageous to find simple, rapidly accessible inhibitors that would be selective for the BET family as exemplified by BRD4 over proteins containing similar bromodomains, such as CBP.

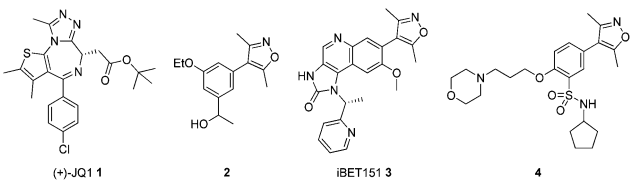
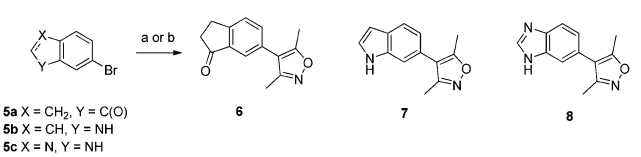


Fig. 1 BET bromodomain inhibitors.

It was thought that fusing a 5-membered ring to the 4-aryl-3,5-dimethylisoxazole moiety of compound **2** (ref. 10) would give access to previously unexploited substitution patterns in known isoxazole-containing bromodomain inhibitors. Simple 5,6-bicyclic bromides **5a–c** were transformed into isoxazoles by either direct arylation of 3,5-dimethylisoxazole or Suzuki reaction of the isoxazolylboronic acid to give compounds **6–8** (Scheme 1).[10,13] When tested in an AlphaScreen® assay using isolated bromodomains, compounds **6** and **7** were modest inhibitors of the first bromodomain of BRD4 (BRD4(1)) with no affinity for the CBP bromodomain whereas compound **8** had comparable affinity for both bromodomains (Table 1).[14]



Scheme 1 Synthesis of indanone, indole and benzimidazole containing inhibitors. *Reagents and conditions*: (a) **5a**, 3,5-dimethylisoxazole, $PdCl_2$, KOAc, *N*,*N*-dimethylacetamide, 130 °C, 50%; (b) 3,5-dimethylisoxazolylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DMF, H_2O, 140 °C μwave, **5b** (12% yield) or **5c** (13% yield).

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‡ Electronic supplementary information (ESI) available: Synthetic details for selected compounds and crystallographic data for compound **15**. See DOI: 10.1039/c2md20189e

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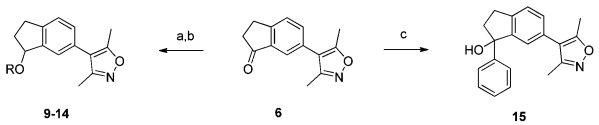
Table 1 Inhibition of BRD4 and CBP bromodomains by target compounds in AlphaScreen®

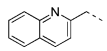
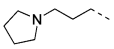
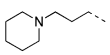
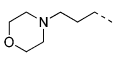
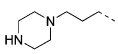
	pIC_{50}[a]	
Cpd	BRD4(1)	CBP
6	4.2 ± 0.3 (2)	<4.6 (2)
7	4.7 ± 0.2 (4)	<5.0 (2)
8	5.2 ± 0.1 (9)	5.4 ± 0.3 (3)

[a] Mean pIC_{50} ± standard error of the mean (number of determinations).



Scheme 2 Derivitization of indanone **6**. *Reagents and conditions*: (a) $NaBH_4$, EtOH, 90%; (b) $ArCH_2Br$, NaH, 76–81% or (i) $Br(CH_2)_3OH$, *p*TsOH, CH_2Cl_2, reflux, 45%; (ii) 2° amine, TEA, THF, reflux, 22–100%; (c) Mg, PhBr, Et_2O, 60%.

Table 2 BRD4(1) and CBP bromodomain affinity of indanes **9–15** in a peptide displacement AlphaScreen® assay

		pIC_{50}[a]	
Cpd	R	BRD4(1)	CBP
9	Bn	4.5 ± 0.1 (4)	<5.0 (2)
10		4.7 ± 0.1 (2)	ND
11		5.2 ± 0.4 (2)	ND
12		5.2 ± 0.5 (2)	ND
13		5.2 ± 0.5 (2)	ND
14		5.4 ± 0.5 (2)	ND
15	NA	5.9 ± 0.1 (6)	4.7 ± 0.2 (3)

[a] Mean pIC_{50} ± standard error of the mean (number of determinations).

The indanone **6** presented an attractive intermediate for further derivitization (Scheme 2). Reduction to the racemic indanol followed by alkylation with benzyl bromide or 2-bromomethyl quinolone gave compounds **9** and **10**. The amines **10–14** were prepared by S_N1 alkylation of the indanol with 3-bromo-*n*-propanol followed by bromide substitution. The basic centres of varying pK_a in compounds **10–14** were designed with the potential to interact with an acidic residue on the edge of the BRD4(1) binding pocket, D145.

Addition of an *O*-benzyl group in compound **9** did not increase the affinity for either BRD4(1) or CBP bromodomains compared to the indanone **6** (Table 2). In this series only the piperazine derived compound **14** with the piperazine group was more potent than compound **9**. A large increase in BRD4(1) affinity, without a corresponding increase in CBP affinity, was

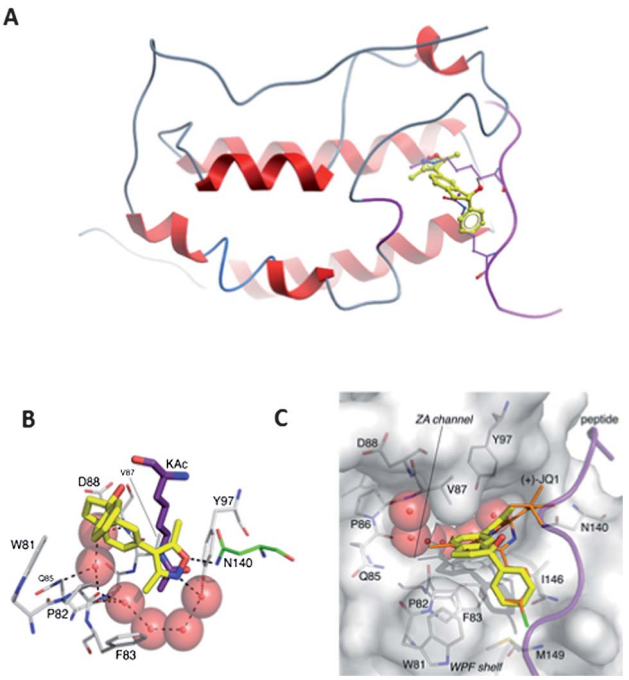


Fig. 2 Compound **15** bound to BRD4(1). (A) Overlay of compound **15** (yellow, PDB ID: 4GPJ) with H4K5AcK8Ac (purple, PDB ID: 3UVW);[1] (B) residues and conserved waters in BRD4(1) binding to compound **15**; (C) surface view of BRD4(1) in the protein–ligand complex overlaid with (+)-JQ1 (orange, PDB ID: 3MXF).

obtained when the indanone **6** was reacted with phenylmagnesium bromide to give the racemic tertiary indanol **15** (Scheme 2).

The increase in affinity of compound **15** for BRD4(1) was explained when it was co-crystallized with this bromodomain (Fig. 2). As expected from previous X-ray structures of BRD4(1)-bound isoxazole ligands,[6,10] the isoxazole oxygen atom formed one hydrogen bond to the conserved asparagine (N140) and the nitrogen atom formed a water-mediated hydrogen bond to Y97. The aryl ring occupied the hydrophobic groove of the WPF shelf, defined by a W81, M149 and I146. Interaction with these three hydrophobic residues is important for the affinity of all known BET inhibitors (Fig. 1).

Encouraged by the excellent potency, selectivity and simple synthesis of indanol **15**, further effort was made to optimize the compound. A range of substituted aryl Grignard reagents were reacted with the ketone, but yields were generally low and the products appeared to decompose during or following isolation. Re-examination of stock solutions of the first indanol **15** by LCMS showed decomposition of the parent compound and a mass loss of 18 amu from the molecular ion peak. It is likely that the doubly benzylic tertiary alcohols dehydrate to give the indenes **16** (Scheme 3).[15]

In an effort to prepare compounds that were as potent as **15** but without the chemical instability problem, attention returned to the benzimidazole **8**. To mimic the potency enhancement of **15** compared to **6**, an aryl group was added to *N*1 of the benzimidazole (Scheme 4). Ullman coupling with phenyl iodide gave a mixture of arylated benzimidazoles **17a** and **17b** that could be

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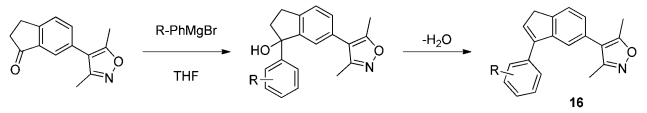
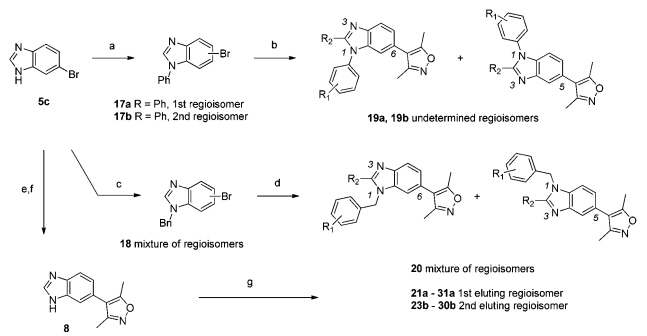
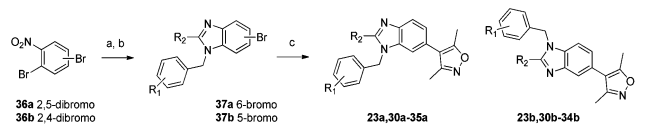


Scheme 3 Decomposition of aryl indanols.



Scheme 4 Synthesis of substituted benzimidazoles. *Reagents and conditions*: (a) PhI, KOH, TBAB, CuI, 110 °C 44% **17a** and 22% **17b**; (b) 3,5-dimethylisoxazolylboronic acid, $Pd(dppf)Cl_2$, $NaHCO_3$, DME, 120 °C, 50% (**19a**) or 26% (**19b**); (c) BnBr, K_2CO_3, MeCN, reflux, 52%; (d) 3,5-dimethylisoxazole, $PdCl_2$, KOAc, *N*,*N*-dimethyl-acetamide, 120 °C, 52% (**20**); (e) Boc_2O, TEA, CH_2Cl_2, 100%; (f) 3,5-dimethylisoxazolylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, 1,4-dioxane, reflux, 48%; (g) RBr, K_2CO_3, MeCN, reflux, 18–74%.



Scheme 5 Regioselective synthesis of benzimidazoles. *Reagents and conditions*: (a) $R_1PhCH_2NH_2$, Na_2CO_3, EtOH, reflux 18–75%; (b) Fe, HCO_2H or $(MeO)_3CMe$, 33–76%; (c) 3,5-dimethylisoxazolylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, 1,4-dioxane, reflux, 22–43%.

separated although the identity of the regioisomers could not be confirmed. The benzyl substituted compounds **18** were prepared in a similar manner by alkylation of *N*1 with benzyl bromide but could not be separated. Isoxazole coupling furnished the first substituted benzimidazoles **19a,b** and **20**. The mixture of regioisomers **20** was tested against both BRD4(1) and CBP and was shown to be at least three-fold more potent than the either of the directly arylated compounds **19a** or **19b**.

Analogues of **20** were prepared using the non-selective alkylation chemistry (**21a–31a, 23b–30b**). By coupling the isoxazole group first, diversification was possible in the last step. The low yield of compound **8** in Scheme 1 was improved by Boc-protection of the benzimidazole followed by Suzuki reaction and *in situ* deprotection. In the case of the substituted benzyl compounds separation was achieved by silica gel chromatography. Comparison of the ^{1}H-NMRs of the first and second eluting regioisomers showed consistent chemical shift changes between the two isomers. This observation confirmed that if the absolute identity of a single regioisomeric pair could be determined, the structures of the others could be correlated by elution order from silica gel.[16]

Table 3 Inhibition of BRD4 and CBP bromodomains by benzimidazoles in a peptide-displacement AlphaScreen® assay

			pIC_{50}[a]		
Cpd	R1	R2	BRD4(1)	CBP	Route[c]
19a[b]	H	H	5.0 ± 0.3 (3)	5.4 ± 0.1 (2)	NA
19b[b]	H	H	5.5 ± 0.2 (2)	<4.6 (2)	NA
20	H	H	6.0 ± 0.1 (2)	ND	NA
21a	3-F	H	6.2 ± 0.1 (2)	4.9 ± 0.3 (2)	sep
22a	3-Cl	H	5.9 ± 0.1 (2)	4.7 ± 0.4 (2)	sep
23a	4-Cl	H	6.1 ± 0.1 (9)	<4.7 (2)	sep/reg
23b	4-Cl	H	5.6 ± 0.1 (2)	5.7 ± 0.2 (2)	sep/reg
24a	3-Br	H	6.0 ± 0.1 (3)	<5.0 (2)	sep
24b	3-Br	H	5.8 ± 0.4 (2)	5.6 ± 0.3 (3)	sep
25a	4-MeO	H	6.1 ± 0.3 (2)	<5.0 (2)	sep
26a	2-Cl	H	6.1 ± 0.5 (2)	<5.0 (2)	sep
27a	$2\text{-}NO_2$	H	6.2 ± 0.4 (2)	<4.0 (2)	sep
27b	$2\text{-}NO_2$	H	4.9 ± 1.0 (2)	4.8 ± 0.9 (3)	sep
28a	4-CN	H	6.7 ± 0.3 (4)	<4.0 (2)	sep
29a	2-CN	H	6.0 ± 0.6 (2)	5.9 ± 0.5 (2)	sep
30a	$3,4\text{-}Cl_2$	H	5.4 ± 0.2 (2)	<4.0 (2)	reg
30b	$3,4\text{-}Cl_2$	H	5.0 ± 0.2 (2)	5.9 ± 0.0 (2)	reg
31a	$4\text{-}CO_2Me$	H	5.9 ± 0.0 (2)	<4.0 (2)	sep
32a	$3\text{-}CON(Me)_2$	H	6.2 ± 0.3 (3)	5.2 ± 0.2 (2)	reg
33a	$4\text{-}CON(Me)_2$	H	5.7 ± 0.1 (2)	5.2 ± 0.2 (2)	reg
34b	4-OH	Me	5.7 ± 0.1 (2)	5.1 ± 0.7 (2)	reg
35a	4-MeO	Me	5.8 ± 0.2 (2)	4.6 ± 0.3 (2)	reg

[a] Mean pIC_{50} ± standard error of the mean (number of determinations). [b] The regioisomeric identity of compounds **19a** and **19b** could not be confirmed. [c] Synthetic route sep: separation of regioisomers as per Scheme 4; reg: regioselective synthesis as per Scheme 5.

A regioselective route to both *N*-benzylbenzimidazoles was developed in order to determine the identity of the regioisomers and prepare additional analogues.[17] Substitution of 2,4- and 2,5-dibromonitrobenzene **36a** and **36b** with benzyl amines was followed by reduction of the nitro group and cyclization to the benzimidazoles **37a** and **37b** with formic acid. Coupling of the 3,5-dimethylisoxazole group completed the synthesis. Compounds **23a**, and **23b** were synthesized by both non-selective and regioselective routes in order to correlate the elution order with the identity of the regioisomers.[18]

Table 3 summarizes the affinity data for the target compounds against BRD4(1) and CBP. In general all compounds were more potent against BRD4(1) than CBP. The 6-isoxazolyl-benzimidazoles were more potent against BRD4(1) than the 5-substituted

Table 4 DSF thermal shift of bromodomains induced by compound **28a**

Bromodomain	T_m shift[a] °C
BRD4(1)	3.2
CBP	1.1
ATAD2	−0.1
BRD9	0.1
PB1(5)	−0.1
PCAF	−0.2
PHIP(2)	−0.4
TAF1(2)	0.0
TIF1α	−0.2

[a] Compound concentration 10 μM, protein concentration 2 μM.

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(see compounds **23a,b**, **24a,b**, **27a,b** and **30a,b**). This observation is consistent with an expected binding mode where the benzyl group fills the same pocket as the phenyl group of compound **15**. The opposite is true of CBP potency, where the 5-substitued regioisomers were more potent. The nature of the benzyl substituent did not have a dramatic effect on the potency with both polar compounds (**25a**, **27a**, **28a**, **31a–33a**) and non-polar compounds (**21a–24a**, **26a**, **30a**) showing similar potency. A methyl group was incorporated at the 2-position of the benzimidazole but it had little effect on either BRD4(1) or CBP potency as shown by comparing compounds **25a** and **35a**.

The most potent inhibitor **28a** was further screened against a panel of diverse bromodomains using a differential scanning fluorimetry (DSF) assay (Table 4).[14] Compound **28a** showed no stabilization of seven other bromodomains and only minimal stabilization of CBP, confirming its selectivity for BRD4(1).

Conclusions

Simple 3,5-dimethylisoxazole substituted benzimidazoles are potent and selective inhibitors of the first bromodomain of BRD4 over the bromodomain of CREB-binding protein. The most potent compound, **28a**, has a BRD4(1) pIC_{50} of 6.7 (IC_{50} = 180 nM) and is at least 100-fold selective over CBP. The addition of the bicyclic benzimidazole ring system to the previously reported phenylisoxazole (compound **2**) has improved both potency for BRD4(1) and selectivity over CBP without loss of selectivity over other bromodomains. With a simple two-step synthesis and regioisomer separation, or three-step regioselective synthesis and multiple positions for modification, this is an attractive template for bromodomain lead discovery projects.

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15 Although the identity of compound **15** as the active compound in the AlphaScreen® assay conditions could not be unequivically confirmed, the presence of the compound in the co-crystal structure with BRD4(1) is evidence for its role as the pharmacologically active component.

16 ^{1}H-NMR resonance differences of the two isoxazole methyl groups and the benzylic methylene were consistently

shifted in all pairs of regioisomers and were used to make compound assignments.

17 E. H. Sessions, M. Smolinski, B. Wang, B. Frackowiak, S. Chowdhury, Y. Yin, Y. T. Chen, C. Ruiz, L. Lin, J. Pocas, T. Schröter, M. D. Cameron, P. LoGrasso, Y. Feng and T. D. Bannister, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 1939–1943.

18 Final compounds were purified by flash chromatography (25 : 1 CH_2Cl_2/EtOAc). Correlating the identity of compounds **23a**, **23b**, **30a** and **30b** made *via* the separation and regioselective routes showed that the 6-isoxazolyl-substituted compounds **23a** and **30a** eluted before 5-isoxazolyl-substituted compounds **23b** and **30b**.

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