

# GSK973 Is an Inhibitor of the Second Bromodomains (BD2s) of the Bromodomain and Extra-Terminal (BET) Family

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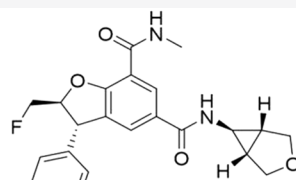
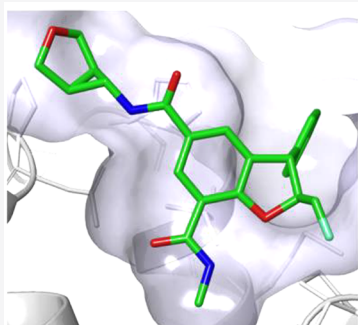
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Supporting Information



**36 (GSK973)**

BRD4 BD2 pIC<sub>50</sub>: 7.8

BRD4 BD1 pIC<sub>50</sub>: 4.6

selectivity BD2/BD1: 1600 fold

Fpo (rat, dog): 48%, 87%

**ABSTRACT:** Pan-BET inhibitors have shown profound efficacy in a number of *in vivo* preclinical models and have entered the clinic in oncology trials where adverse events have been reported. These inhibitors interact equipotently with the eight bromodomains of the BET family of proteins. To better understand the contribution of each domain to their efficacy and to improve from their safety profile, selective inhibitors are required. This Letter discloses the profile of GSK973, a highly selective inhibitor of the second bromodomains of the BET proteins that has undergone extensive preclinical *in vitro* and *in vivo* characterization.

**KEYWORDS:** Bromodomain, BET, constrain, selectivity, dihydrobenzofuran, fluorine

The bromodomain and extra-terminal (BET) family of proteins are known as epigenetic readers of chromatin. Indeed, the two tandem bromodomains (BD1 and BD2) of each of the four proteins (BRD2,3,4, and T) recognize and bind to specific acetylated-lysine residues of histone tails and non-histone proteins and play a major role in the regulation of gene transcription (ref 1). Pan-BET inhibitors have shown profound efficacy in a number of preclinical *in vitro* and *in vivo* models (ref 2) with several molecules progressed into the clinic. A number of adverse events have been reported in oncology trials to date (ref 3), in particular thrombocytopenia. Various strategies (dosing holidays, combination therapy) have been put in place in an attempt to circumvent these safety concerns and maximize the potential of this mechanism.

Pan-BET inhibitors interact equipotently with the eight bromodomains of the BET family. In order to elucidate the functional contribution of each bromodomain to the phenotype observed with pan-BET inhibitors and with the ambition of exploring whether the efficacy and toxicology effects observed could be dissociated by selective bromodomain targeting, we aimed to identify selective small molecule inhibitors of BD2s. The high homology between the 4 BD1s

and the 4 BD2s (see the [Supporting Information](#)) makes the identification of isoform selective inhibitors (interacting only with one or two BD of a given protein) difficult. Indeed, an HTS performed at GSK only identified hits either equipotent at all BDs or biased against all BD1 or all BD2 BDs (ref 4).

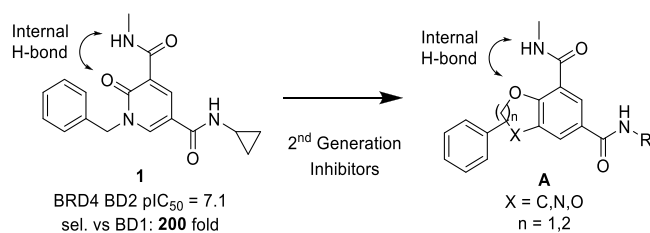
We (refs 4–6) like others (refs 7 and 8) have recently disclosed the identification of drug-like selective pan-BD2 inhibitors. In particular, we have disclosed the profile of a series of pyridone derivatives with excellent selectivity for BD2s over BD1s (ref 4). The pyridone series was characterized by a methyl amide KAc mimetic, stabilized in its bound conformation by an internal hydrogen bond; a benzylic substituent projected toward the BET WPF shelf and a secondary amide (cyclopropyl amide in the case of **1**, [Figure 1](#))

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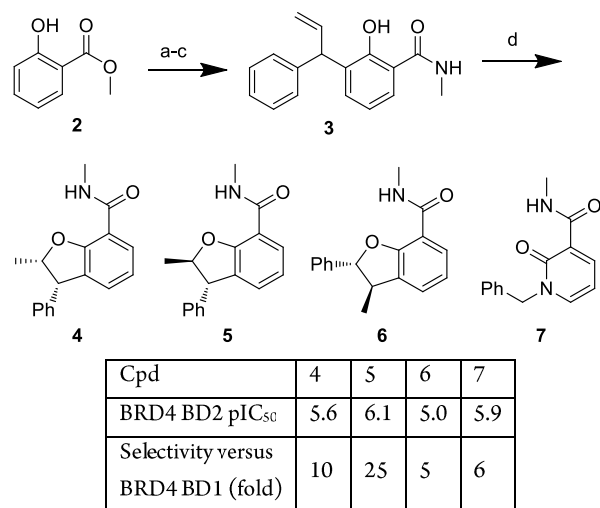




**Figure 1.** Strategy to increase potency and selectivity.

which proved to increase both potency and selectivity thanks to an additional hydrogen bond with Asn429 (BRD4 BD2 numbering) and better shape complementary for BD2 compared to BD1. This series delivered drug-like molecules with good potency and BD2/BD1 selectivity (in most cases >100-fold). However, to fully interrogate the properties of domain selectivity, we were interested in identifying novel series with similar potency (FRET BRD4 BD2  $pIC_{50} > 7$ ) but increased level of BD2/BD1 selectivity (>1000-fold). Crystallographic data suggested that constraining the WPF shelf substituent by cyclization of the benzylic position should mimic the bound conformation of **1**, which may have a positive impact on both potency and selectivity. In order to keep the internal H-bond known to provide enhanced potency, bicyclic cores **A** were considered (Figure 1).

Dihydrobenzofurans (DBFs) could be readily accessed to test this hypothesis (Figure 2). Alkylation of **2**, Claisen rearrange-

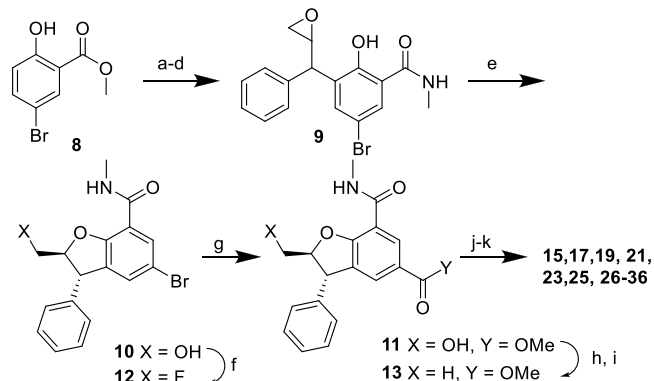


**Figure 2.** Synthesis and comparison of substituted dihydrobenzofuran cores. Reagents and conditions: (a) Cinnamyl chloride,  $K_2CO_3$ , KI, acetone, reflux, 88%; (b) *N,N*-dimethylaniline, reflux, 55%; (c)  $MeNH_2$ , THF, room temperature, 91%; (d) TFA, reflux, 6–16%.

ment, and amide formation led to **3** which could be cyclized using refluxing TFA to afford racemic compounds **4–6** in low yields (6–16%) (**6** is obtained via a Wagner–Meerwein rearrangement). Among the three derivatives isolated, compound **5** appeared to be the most interesting in terms of both potency and selectivity and compared favorably with the profile of fragment **7**, which had led to **1**.

The synthetic route to access functionalized analogues of **5** is shown in Scheme 1. Epoxide **9** could be obtained from **8** using the same Claisen rearrangement as that for **3**, followed by epoxidation using *m*-CPBA. Interestingly, **9** was obtained as a 1:1 mixture of diastereoisomers, but the cyclization to **10**

### Scheme 1. Synthesis of Functionalized DBFs<sup>a</sup>



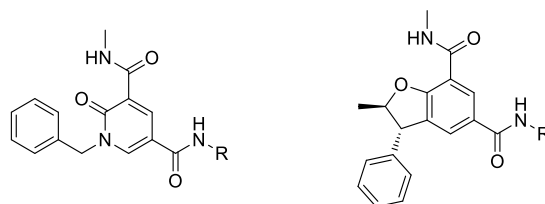
<sup>a</sup>Reagents and conditions: (a) Cinnamyl chloride,  $K_2CO_3$ , KI, acetone, reflux, 100%; (b) *N,N*-dimethylaniline, reflux; (c)  $MeNH_2$ , THF, room temperature, 56% (two steps); (d) *m*-CPBA,  $CH_2Cl_2$ , 0 °C, 98%; (e) KOH, DMSO/ $H_2O$ , 0 °C, 67%; (f) Deoxofluor,  $CH_2Cl_2$ , 0–40 °C, 71%; (g)  $Pd(OAc)_2$ , Xantphos, CO, MeOH, DMF, 70 °C, 49%; (h)  $I_2$ ,  $PPh_3$ , imidazole,  $CH_2Cl_2$ , rt, 79%; (i)  $NEt_3$ ,  $Pd/C$ ,  $H_2$  (1 atm), MeOH, rt, 56%; (j) LiOH or NaOH, MeOH, THF,  $H_2O$ , rt, >95%; (k)  $RNH_2$ , HATU,  $NEt_3$ , rt, 13–92%.

under mild basic conditions led only to the more thermodynamically stable *trans*-DBF (*dr* > 95:5) thanks to an *in situ* epimerization of the dibenzylic position. From **10**, a carbonylation led to ester **11**. It was also possible to substitute the primary hydroxyl of **10** by a fluorine (**12**) or reduce it to the corresponding alkane (**13**). Saponification of the ester followed by amide coupling provided compounds **14–36**. Separation of the enantiomers by chiral chromatography could be done at any stage once **10** was obtained. One enantiomer was always significantly more potent than the other and when cocrystallized with the bromodomain construct always had the stereochemistry as drawn in the tables and scheme. We therefore considered that the most potent BD2 activities always resided in this isomer. Data provided below are for single enantiomers unless otherwise stated.

As can be seen in Table 1, the DBF series was more potent and selective than the pyridone series: For most pairs presented (**14–21**), the DBF analogue was at least 3-fold more potent against BRD4 BD2 than its pyridone equivalent with excellent ligand efficiency ( $LE > 0.4$ ) for both series when lipophilic amines were used. The selectivity was superior in the DBF series by virtue of not only higher potency against BD2 but also lower potency against BD1. Excellent selectivity (>1000-fold) could be achieved with small amide substituents (compounds **19** and **21**), but this was to the detriment of lipophilicity which was systematically increased by at least 1.1 log units compared to the equivalent pyridone. Introducing polarity in the amide substituent was detrimental to activity (compounds **22–25**).

Compound **21** was considered as the lead molecule and further profiled. As can be seen in Table 2, the low molecular weight and moderate lipophilicity were properties affording good passive permeability and consequently good cell potency, as seen by inhibition of monocyte chemoattractant protein-1 (MCP-1) in a lipopolysaccharide (LPS)-stimulated human peripheral blood mononuclear cell (PBMC) assay. Moderate protein binding aided the retention of high potency for inhibition of MCP-1 release in an LPS-stimulated human whole blood assay. High throughput solubility was moderate.

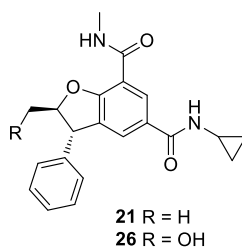
Table 1. Comparison of the Pyridone and DBF Series Activities



| Cpd | series <sup>b</sup> | R  | BRD4 pIC <sub>50</sub> <sup>a</sup> |      | fold selectivity | ChromLogD @ pH 7.4 |
|-----|---------------------|--|-------------------------------------|------|------------------|--------------------|
|     |                     |  | BD2 (LE)                            | BD1  |                  |                    |
| 14  | pyridone            | H  | 6.5 (0.42)                          | 5.1  | 25               | 2.03               |
| 15  | DBF                 |  | 7 (0.42)                            | 4.8  | 160              | 3.28               |
| 16  | pyridone            | methyl   | 6.9 (0.43)                          | 5.1  | 60               | 2.38               |
| 17  | DBF                 |  | 7.5 (0.43)                          | 4.9  | 400              | 3.55               |
| 18  | pyridone            | ethyl  | 6.9 (0.41)                          | 4.8  | 125              | 2.99               |
| 19  | DBF                 |  | 7.4 (0.41)                          | 4.4  | 1000             | 4.11               |
| 20  | pyridone            | c-Pr   | 7.1 (0.41)                          | 4.8  | 200              | 3.07               |
| 21  | DBF                 |  | 7.9 (0.42)                          | 4.7  | 1600             | 4.24               |
| 22  | pyridone            | CH <sub>2</sub> CH <sub>2</sub> OH               | 6.2 (0.35)                          | 4.7  | 30               | 1.9                |
| 23  | DBF                 |  | 6.8 (0.36)                          | <4.3 | >320             | 3.12               |
| 24  | pyridone            | CH <sub>2</sub> CH <sub>2</sub> OCH <sub>3</sub> | 6.2 (0.34)                          | 5.1  | 13               | 2.74               |
| 25  | DBF                 |  | 6.7 (0.34)                          | 4.4  | 200              | 3.81               |

<sup>a</sup>BRD4 potencies are representative of the potencies against the BET proteins (see the Supporting Information for full profiling). <sup>b</sup>See ref 4 for all data on the pyridone series.

Table 2. Characterization of Compounds 21 and 26



| compound                                     | 21                | 26                |
|--|-------------------|-------------------|
| MW, cLogP, ChromLogD                         | 350.4; 2.88; 4.24 | 366.4; 1.41; 2.53 |
| AMP permeability (nM/s)                      | 440               | 266               |
| CLND solubility (μg/mL)                      | 137               | >177              |
| HSA binding (%)                              | 89                | 79                |
| h PBMC pIC <sub>50</sub> (MCP-1)             | 8.2               |                   |
| h whole blood pIC <sub>50</sub> (MCP-1)      | 6.7               | 6.1               |
| hepatocyte Cli (rat, human, mL/min/g tissue) | 2.48; 1.42        | <0.8; <0.45       |
| rat PK (n = 1, 1 mg/kg IV)                   |                   |                   |
| CL (mL/min/kg) (% LBF)                       | 90 (>100%)        | 74 (65%)          |
| Cl <sub>renal</sub> (mL/min/kg)              | 0.2               | 23                |
| V <sub>ss</sub> (L/kg)                       | 2.3               | 1.4               |
| T <sub>1/2</sub> (h)                         | 0.4               | 0.4               |
| rat PK (n = 3, 3 mg/kg po)                   |                   |                   |
| Fpo (%)                                      |                   | 2                 |

Unfortunately, the compound showed moderate turnover *in vitro* in rat and human hepatocytes. *In vivo*, in the rat, this translated to high blood clearance. *In vivo* clearance was assumed to be metabolic as renal clearance was minimal. The compound was distributed into tissues, but the high clearance resulted in a short elimination half-life unacceptable for further development as an oral therapeutic.

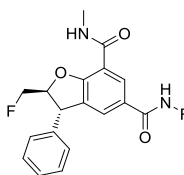
We considered that reducing the lipophilicity in this series could improve not only pharmacokinetics but also have a



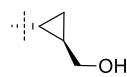
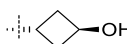

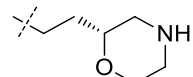
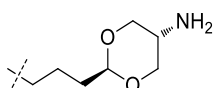
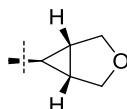
beneficial impact on other developability properties such as solubility. However, retaining low molecular weight and ligand efficiency was essential. As compounds 23 and 25 (Table 1) had shown that small polar substituents were poorly tolerated in the amide region and as chemistry enabled further functionalization of the C2 position, we assessed the impact on the potency and lipophilicity of a small polar substituent in this position. Hydroxyl and fluorine were considered, knowing that the latter also reduces lipophilicity when attached to an alkyl group (refs 9–11).

Compound 26 (Table 2) is a representative example of the C2-hydroxyl derivatives that were prepared. It is less potent (BD2 pIC<sub>50</sub> = 7.4) than 21 but remains selective (BD1 pIC<sub>50</sub> = 4.5, selectivity 800-fold). Lipophilicity is significantly reduced, but despite increased free fraction, 26 is significantly less potent in human whole blood (pIC<sub>50</sub> of 6.1 versus 6.7 for 21). Surprisingly, despite the low turnover in rat hepatocytes, the compound was still rapidly cleared *in vivo* in rats. Metabolic clearance was somewhat reduced, in line with lower hepatocyte clearance, but an increased renal clearance was observed. This was likely a resulting effect of the reduced ChromLogD altering filtration and/or secretion mechanisms. The elimination half-life remained short and oral bioavailability very low. Overall, the drop in potency and the poor *in vivo* pharmacokinetics of 26 despite low ChromLogD led us to put work on this subseries on hold and focus on the C2-fluoro derivatives.

The profile of a number of representative examples of C2-fluoro inhibitors is presented in Table 3. The chemical complexity of introducing a substituent on the C3 phenyl ring to tune intrinsic properties led us to focus on modifying the C5-amide. We also knew from the pyridone series that substituting the C3-phenyl ring with electron withdrawing or low LogD substituents was usually detrimental to potency (ref 4).

Table 3. Profile of C2-Fluoro Inhibitors



| Cpd  | R   | BRD4 pIC <sub>50</sub> |              | Fold Selectivity | hWB pIC <sub>50</sub> | ChromLogD @ pH 7.4 | Hepatocyte Clearance (mL/min/g tissue) |        |        |
|------|---|------------------------|--------------|------------------|-----------------------|--------------------|--|--------|--------|
|      |   | BD2 (LE)               | BD1          |                  |                       |                    | Rat                                    | Dog    | human  |
| 27   | H   | 6.9 (0.39)             | 4.7          | 160              | -                     | 2.89               | -                                      | -      | -      |
| 28   | CH <sub>3</sub>   | 7.5 (0.41)             | 4.9          | 400              | 6.7                   | 3.34               | 3.02                                   | < 1.26 | < 0.45 |
| 29   |    | 7.6 (0.39)             | <3.3         | >20000           | 6.7                   | 3.94               | 3.2                                    | < 1.26 | < 0.45 |
| 30*  |    | 8.0 (0.39)             | 5.0          | 1000             | 7.0                   | 4.53               | -                                      | -      | -      |
| 31*  |    | 7.7 (0.36)             | 4.8          | 800              | 6.7                   | 3.00               | 1.65                                   | <0.65  | <0.45  |
| 32   |   | 7.1 (0.34)             | 4.5          | 400              | 6.5                   | 2.92               | 1.84                                   | -      | <0.45  |
| 33** |  | 6.8 (0.32)             | <4.3         | >300             | -                     | 3.51               | -                                      | -      | -      |
| 34*  |  | 7.4 (0.32)             | 4.3 (1/5)*** | 1200             | 6.8                   | 2.18               | 1.28                                   | <0.65  | <0.45  |
| 35   |  | 7.6 (0.31)             | 4.8          | 600              | 6.9                   | 2.94               | 1.39                                   | <0.65  | <0.45  |
| 36   |  | 7.8 (0.36)             | 4.6          | 1600             | 7.3                   | 3.56               | 1.76                                   | <0.65  | <0.45  |

<sup>a</sup>The R group is a single enantiomer. <sup>b</sup>The R group is a 1:1 mixture of epimers.

Table 4. *In Vivo* Pharmacokinetics of a Set of C2-Fluoro DBFs

| compound                 |                                 | 31               | 35               | 36               |
|--------------------------|---------------------------------|------------------|------------------|------------------|
| AMP (permeability, nM/s) |                                 | 73               | 45               | 165              |
| CLND solubility (μg/mL)  |                                 | 114              | 174              | 173              |
| species                  |                                 | rat <sup>a</sup> | rat <sup>a</sup> | rat <sup>b</sup> |
| IV PK                    | CL (mL/min/kg) (% LBF)          | 98 (120%)        | 73 (81%)         | 73 (89%)         |
|                          | CL <sub>renal</sub> (mL/min/kg) | 4                | 10               | 4                |
|                          | V <sub>ss</sub> (L/kg)          | 1.7              | 2.1              | 2.1              |
|                          | T <sub>1/2</sub> (h)            | 0.5              | 0.6              | 0.6              |
| oral PK                  | Fpo (%)                         | 15               | 3                | 48               |
|                          |                                 |                  |                  | 87 <sup>d</sup>  |

<sup>a</sup>*n* = 1, 1 h IV infusion at 1 mg/kg, *n* = 3 PO gavage at 3 mg/kg. <sup>b</sup>*n* = 3, 1 h IV infusion at 1 mg/kg, *n* = 3 PO gavage at 3 mg/kg. <sup>c</sup>*n* = 3, 1 h IV infusion at 1 mg/kg, *n* = 2 PO gavage at 2 mg/kg. <sup>d</sup>Wet Bead-milled Spray Dried material (EXP109376) formulated in 1.5% HPMC PHARMACOAT 603:0.15% (w/v) sodium lauryl sulfate (aq) (1.5: v/v).

The C2-fluoro derivatives were potent and selective with the cyclopropyl derivative **29** providing excellent selectivity to the detriment of lipophilicity (compare **29** with **27** and **28**, Table 3). Overall, the ChromLogD values of the C2-fluorine derivatives were lower than their C2-methyl analogue (compare **29** and **21** as examples). Crystallographic data (not shown) suggested that the introduction of a specific methyl substituent on the cyclopropyl ring of **29** could increase van der Waals interactions with the protein, and this proved indeed the case with compound **30** being significantly more potent than **29**. It was possible to reduce the lipophilicity by introduction of a hydroxyl (compound **31**). All other attempts to introduce substituents balancing potency and selectivity in a reasonable LogD space were unsuccessful (see compounds **32** and **33** as examples). Another opportunity to lower lipophilicity was to introduce basicity on the chain, which was feasible as long as the basic nitrogen was far enough from the amide to be solvent exposed (compounds **34** and **35**) (ref 12). From the cyclopropyl motif, it was also possible to increase selectivity and lower lipophilicity with appropriate ether derivatives (compound **36**) (ref 13). Overall, *in vitro* potency translated into good human whole blood potency (hWB) with **36** being one of the most potent and selective compounds made.

Regarding *in vitro* PK, the series showed a marked difference between species with most compounds being more metabolically stable in dog and human hepatocytes than rat. Table 4 describes the *in vivo* rat PK data for a set of optimized compounds. As can be seen, in most cases, high *in vitro* clearance translated to high *in vivo* clearance and, as a result, limited oral bioavailability in the rat when tested. Compound **36** was deemed worth progressing further thanks to its excellent *in vitro* profile and acceptable exposure in rat. Indeed, it showed excellent pharmacokinetics in dog with low blood clearance (in agreement with the hepatocyte data), good oral bioavailability, and a moderate half-life. The correlation between *in vitro* and *in vivo* metabolic clearance provided confidence that low metabolic clearance could be achieved in humans.

Compound **36** (GSK973) was further profiled. It showed acceptable FaSSIF solubility from crystalline material (80  $\mu\text{g}/\text{mL}$ ) and no cardiac liability *in vitro* (hERG  $\text{pIC}_{50} < 4.3$ ), and it was negative in the AMES and mouse lymphoma assays (MLA), a glutathione (GSH) trapping, and CYP3A4 time dependent inhibition (TDI) assays. The selectivity profile against the other bromodomains was assessed using BROMOScan (Figure 3, details in the Supporting Information) and showed no significant cross-reactivity (the lowest selectivity was against TAF1 BD2 and was 200-fold). The BD2/BD1 selectivity was confirmed in this assay format as well as by SPR (with  $K_d$  against BRD4 BD2 and BD1 of 34 nM and >3000 nM; see the Supporting Information). As a further assessment of possible off-target activity, **36** was screened and proved inactive against an internal panel of 40 targets considered as potential liabilities (data not shown). The enantiomer of GSK973, with BD1/BD2  $\text{pIC}_{50}$ 's of <4.3 and 5.1, represents a good negative control (ref 14).

A crystal structure of compound **36** bound to BRD4 BD2 superimposed with **1** is shown in Figure 4. As intended, both bind to the KAc pocket in a similar way. The carbonyl of the C7 amide of GSK973 hydrogen-bonds to the conserved asparagine Asn429 and via a bridging water molecule to Tyr386. In addition, the NH of the C5 amide makes a second

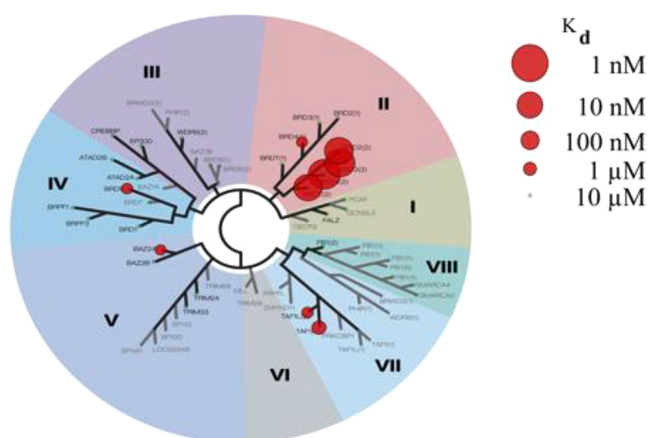


Figure 3. BROMOScan data for compound **36**.

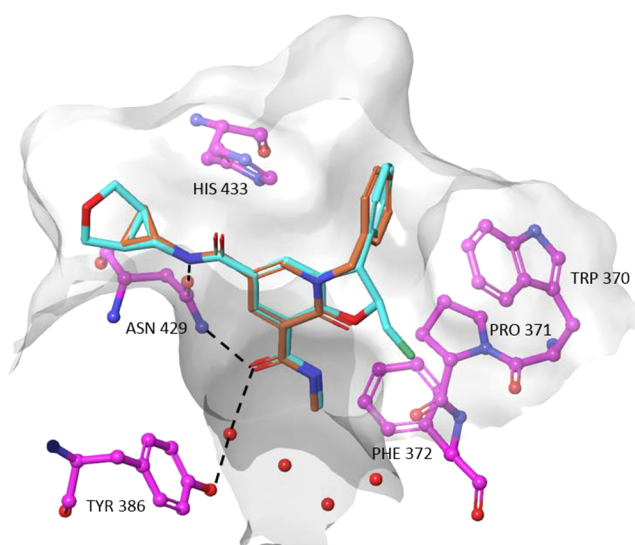


Figure 4. Compounds **1** (blue, PDB: 6zb1) and **36** (brown, PDB 6z8p) bound to BRD2 BD2.

interaction to the carbonyl group of Asn429. The phenyl group in the C3 position sits on the WPF shelf and is involved in three-way edge-to-face aromatic interactions between Trp370 and His433. The fluoromethyl group off the C2 position projects into the ZA channel, between Tyr370 and Leu381.

In conclusion, our strategy of constraining the WPF shelf substituent of the pyridone series using a DBF template in order to obtain more potent and selective pan-BD2 BET inhibitors proved successful. Compound **36** is a drug-like inhibitor with exceptional potency and selectivity. Its *in vitro* profile and good pharmacokinetics in preclinical species make it a useful tool for further probing the separate contributions of the BDs to BET-associated functional phenotypes.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsmchemlett.0c00247>.

Assay and pharmacokinetic study protocols, protein production and crystallographic information, compound synthesis and characterization, and BROMOScan data for **36** (PDF)

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The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

AMP, artificial membrane permeability; BET, bromodomain and extra-terminal; BD, bromodomain; DBF, dihydrobenzofuran; LBF, liver blood flow; LE, ligand efficiency; FaSSiF, fasted state simulated intestinal fluid; hWB, human whole blood; TAF1, transcription initiation factor TFIID subunit 1.

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