

Angewandte International Edition Chemie

Epigenetics

International Edition: DOI: 10.1002/anie.201603928 German Edition: DOI: 10.1002/ange.201603928

A Chemical Probe for the ATAD2 Bromodomain

Paul Bamborough,* Chun-wa Chung, Emmanuel H. Demont,* Rebecca C. Furze, Andrew J. Bannister, Ka Hing Che, Hawa Diallo, Clement Douault, Paola Grandi, Tony Kouzarides, Anne-Marie Michon, Darren J. Mitchell, Rab K. Prinjha, Christina Rau, Samuel Robson, Robert J. Sheppard, Richard Upton, and Robert J. Watson

Abstract: ATAD2 is a cancer-associated protein whose bromodomain has been described as among the least druggable of that target class. Starting from a potent lead, permeability and selectivity were improved through a dual approach: 1) using CF_2 as a sulfone bio-isostere to exploit the unique properties of fluorine, and 2) using 1,3-interactions to control the conformation of a piperidine ring. This resulted in the first reported low-nanomolar, selective and cell permeable chemical probe for ATAD2.

igh expression levels of ATAD2 (ATPase family, AAA domain containing 2), also called ANCCA (AAA + nuclear coregulator cancer-associated), correlate with poor outcomes in several cancers, and its knockdown modulates multiple tumor cell growth factors.^[1–3] Efforts to target this protein have focused on competitive binding to the acetyl-lysine (KAc) site of its bromodomain, but the role of the bromodomain in the biology of ATAD2 is unclear. We have developed chemical tools to try to understand this, and recently reported the discovery of quinolinones and naph-thyridones such as 1–3 and 5–7 that bind to the KAc site of ATAD2.^[4,5]

The profound biological effects associated with BET inhibition complicate interpretation of phenotypes observed with unselective inhibitors of other bromodomains.^[6] While potent against ATAD2, naphthyridone **5** had only modest selectivity over the BETs, represented in Table 1 by the first bromodomain of BRD4 (BRD4 BD1). We recently reported

[*] Dr. P. Bamborough, Dr. C. Chung, Dr. E. H. Demont, Dr. R. C. Furze, Dr. H. Diallo, C. Douault, D. J. Mitchell, Dr. R. K. Prinjha, R. J. Sheppard, Dr. R. Upton, Dr. R. J. Watson GlaxoSmithKline Gunnels Wood Road, Stevenage, SG1 2NY (UK) E-mail: Paul.A.Bamborough@gsk.com Emmanuel.H.Demont@gsk.com Dr. P. Grandi, Dr. A.-M. Michon, Dr. C. Rau Cellzome GmbH, GlaxoSmithKline Meyerhofstrasse 1, 69117 Heidelberg (Germany) Dr. A. J. Bannister, Dr. K. H. Che, Prof. T. Kouzarides, Dr. S. Robson Gurdon Institute Tennis Court Road, Cambridge CB2 1QN (UK) R. J. Sheppard Current address: AstraZeneca Milton Road, Cambridge CB4 OWG (UK) Supporting information (X-ray, ITC, assay, molecular modelling,

LNCaP biology, NMR and chemistry supplementary methods) and the ORCID identification numbers for the authors of this article can be found under http://dx.doi.org/10.1002/anie.201603928. **Table 1:** Micromolar lead to nanomolar ATAD2 inhibitors. For statistics see Table S1a, Supporting Information.



selectivity improvements from occupying an electrostatically positive site near the KAc pocket, the RVF shelf, with negative polarity (compare 5 to 6 to see the effect of introducing cyclic sulfones at the C3' position of the piperidine ring with (R,R) stereochemistry).^[5] An X-ray structure of ATAD2 bound to 3 showed that its sulfone oxygen atoms displace a weakly-bound water molecule and accept two hydrogen bonds from the guanidinium group of Arg1077 (Figure 1a). The WPF shelf of the BET bromodomains (the analogous subsite to the ATAD2 RVF shelf) contains lipophilic amino acids Trp81 and Met149 in place of ATAD2 residues Arg1007 and Arg1077, so is less tolerant of the sulfone group's polarity. Compounds with hydrophobic C3'-substituents such as the cyclohexylmethylenes 2 and 5 are more potent against the BETs, which is consistent with the crystal structure of 2 bound to BRD4 BD1, where the C3'cyclohexyl ring rests on the WPF shelf (Figure 1b). Unfortunately, while the sulfone C3' group improved selectivity it also impacted passive permeability, raising doubts over the suitability of 6 and 7 as tool compounds for chromatin-bound ATAD2. Here, we describe the conclusion of our optimization of the series to overcome this.

Alternative C3' functional groups of intermediate polarity had been found inferior to the sulfone in potency or

Angew. Chem. Int. Ed. 2016, 55, 1-6

© 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



Figure 1. A) 2 views of the X-ray structure of 3 bound to ATAD2 (PDB 5a83). B) Superimposed X-ray structures of BRD4 BD1 bound to
1 (orange, PDB 5a5s) and 2 (cyan, PDB 5a85). C) Superimposed X-ray structures of BRD4 BD1 bound to 1 (orange, PDB 5a5s) and 4 (green, PDB 5lj2) showing the di-axial conformation of the piperidine ring. D) ATAD2 crystallographic binding modes of 16 (cyan, PDB 5lj0) and 3 (green, PDB 5a83). E) Binding modes in BRD4 BD1 of 16 (cyan, PDB 5lj1) and 4 (green, PDB 5lj2). For refinement statistics see Table S3; for density maps Figure S6, Supporting Information.

selectivity.^[5] We therefore considered the possibilities offered by fluorine substitution. The effect of fluorine on features of organic molecules such as conformation, pK_a , permeability and metabolism has recently been reviewed.^[7] Fluorine combines unique properties of polarity, lipophilicity and low polarisability.^[8-10] Regarding noncovalent interactions, the ability of organofluorine to participate in hydrogen bonds has been debated.^[10–15] While the lone pairs of organic fluorine seem too tightly held to hydrogen-bond, strong charge–dipole interactions can be formed with cations.^[15]

The bi-lobal negative charge patches characteristic of the molecular surface of the SO₂ group of **3** are well oriented to interact with the ATAD2 RVF shelf residue Arg1077 (Figure 1a). The CF₂ group possesses geometrically similar electronegative patches (Figure S1, Supporting Information) and should be compatible with polar interactions with Arg1077. Indeed, the guanidinium group of arginine has been described as highly fluorophilic.^[14] We reasoned that CF₂ may provide an isosteric replacement for SO₂, that its large dipole moment relative to CH₂ (2.57 D in 1,1-difluorocyclohexane^[16]) might disfavour interaction with the hydrophobic BRD4 BD1 WPF shelf, and that the expected increase in lipophilicity should have a positive effect on permeability.

Fluorinated derivatives 8 and 9 were made according to previously reported procedures^[5] and their profile compared to the direct sulfone analogues 6 and 7 (Table 1). The higher log *D* and lower polar surface area of the difluoromethylene compounds relative to the sulfones resulted in dramatically improved artificial membrane permeability, comparable to the cyclohexyl analogues (compare 5, 6 and 8). The difluoromethylenes 8 and 9 had greater TR-FRET ATAD2 potency than the sulfones (6 and 7), which we rationalize by a decreased desolvation penalty. While the selectivity of 8 and 9 for ATAD2 over BRD4 BD1 was slightly lower than the analogous sulfones 6 and 7, it was significantly better than the cyclohexyl 5 and matched our probe critera of >2 logs. Overall, in this series the CF₂ group proved to be an excellent SO₂ isostere.

In parallel, we sought ways to increase the selectivity window by better understanding the binding mode of analogues of 6 to BRD4 BD1. We assumed that this would be similar to previous analogues such as 1 and 2 (Figure 1b). The naphthyridone binds in the KAc-pocket, with the 3' substituent of 2 located on the WPF shelf as outlined above. We obtained a crystal structure of BRD4 BD1 bound to 4, the amino-pyridine analogue of 6. The bound position of the naphthyridone of 4 is very like that of 1. Unexpectedly, rather than the 3' substituent lying on the WPF shelf as expected by analogy with 2, the piperidine ring of 4 adopts a trans di-axial conformation, positioning the SO₂-containing 3'-substituent in a solvent-exposed position far from the WPF shelf (Figure 1 c). This is in sharp contrast to the trans di-equatorial conformation seen in ATAD2 for sulfone-containing 3'substituted compounds like 3 (Figure 1a). In this axial conformation, the piperidine nitrogen of 4 makes a saltbridge interaction with the sidechain of BRD4 Asp144. However, as this was not seen with 1 or 2 this cannot be the main driver for the adoption of the di-axial conformation in BRD4 BD1. Rather, this conformation allows the polar

www.angewandte.org

© 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

These are not the final page numbers!

sulfone group to evade the hydrophobic WPF shelf environment.

Interestingly, gas-phase ab initio calculations on model systems favor the di-axial conformation, which is stabilised by a 1,3 interaction between the alkoxy oxygen and the protonated piperidine nitrogen (Figure S1c, Supporting Information). In a continuum water model the strong electrostatic contribution was diminished and di-equatorial was slightly preferred. NMR experiments on **4** in water indicated an equilibrium, which was rapid on the NMR timescale between these two piperidine chair conformers, in an approximately 5:2 ratio in favour of the *trans* di-equatorial (see Supporting Information). Hence, both molecular modelling and experimental data support the *trans* di-axial conformer as a low energy form for this structure.

These observations suggested that selectivity for ATAD2 over BRD4 BD1 might be increased by stabilization of the equatorial piperidine conformation over axial. Introduction of a substituent *cis* to the ether at the C5' position would create a 1,3 steric repulsion in the tri-axial conformation, favouring the tri-equatorial. We tested this idea by introducing a methoxy substituent in the C5' position via the route shown in Scheme 1.



Scheme 1. Conditions: a) *m*-CPBA, CH_2CI_2 , 0°C; b) NaH, MeI, DMF, 0°C, 83% (2 steps); c) LiClO₄, NaN₃, CH₃CN, 80°C, 35%; d) tBuOK, THF, 0°C then RCH₂OTf, 70–72%; e) H₂, Pd/C, MeOH, room temperature, 90–91%; f) **14**, tBuONa, BrettPhos, Pd(OAc)₂ or Pd(dba)₂, THF, 60°C, 37–43%; g) NBS, CH₂Cl₂, -10°C, 94–97%; h) ArB(OH)₂, K₂CO₃, Pd(OAc)₂, cataCXium A, dioxane/H₂O, microwave, 100°C, 40–49%; i) TFA, reflux, >93%.

Epoxide **11** was easily obtained as a single diastereoisomer from alcohol **10** and could be opened with sodium azide in the presence of LiClO_4 to give a 1:1 mixture of regioisomers, which were separable.^[17] Following alkylation and reduction of **12**, amines **13 a,b** could be coupled with naphthyridone **14** in moderate yields.^[5] Regioselective bromination, Suzuki coupling and deprotection in acidic conditions provided the inhibitors **15–17**. Chiral purification could be performed at the final stage or following the Buchwald coupling.

Data for conformationally biased analogues are shown in Table 2. The C5'-OMe C3'-sulfone-containing piperidine **15**

Table 2: Comparison of di- and tri-substituted inhibitors. For statistics see Table S1a, Supporting Information.

	6	15	8	16	17
ATAD2 TR-FRET pIC ₅₀	6.9	6.9	7.4	7.3	5.5
ATAD2 Bromosphere pIC ₅₀	7.5	8.4	7.2	8.0	
BRD4 BD1 TR-FRET pIC ₅₀	4.8	3.8	5.2	\leq 4.5	≤4.4
TR-FRET selectivity (logs)	2.1	3.1	2.2	\geq 2.8	≥1.1
Chrom logD (pH 7.4)	1.6	1.7	3.0	3.2	3.1
Polar surface area (Å ²)	126	135	92	101	101
Artificial membrane permeability	< 3	< 3	86	190	156
(пм s ⁻¹ , pH 7.4)					

retained the ATAD2 activity of des-methoxy **6**, but its BRD4 BD1 activity was significantly weaker, resulting in an increased selectivity window of >3 logs. We believe that this is due to the destabilization of the axial conformation favored for BRD4 BD1 binding relative to the equatorial favored by ATAD2. This conclusion was supported by NMR data for **15** in water which indicated a strong preference for the tri-equatorial conformation, with no evidence for significant presence of tri-axial (see Supporting Information).

Encouraged by this, we also made **16**, the equatoriallybiased C5'-OMe analogue of the difluorocyclohexyl **8**. By TR-FRET, this retained the enhanced selectivity of **15**, being equipotent to **8** against ATAD2 with reduced BRD4 BD1 activity (Table 2). As before, replacing the sulfone by difluoromethyl resulted in improved permeability. Compound **17** (the opposite enantiomer of **16**) is significantly weaker against ATAD2, so represents a useful negative control for cellular assays.

Crystal structures were obtained of 16 bound to both ATAD2 and to BRD4 BD1. As expected, in ATAD2 the binding mode of 16 is very similar to that of 3, with the entire molecules closely superimposable (Figure 1 d). The piperidine binds in a tri-equatorial conformation with the C5' methoxy group making no apparent interaction with the protein. The two fluorine atoms are close to the guanidinium terminal nitrogen of Arg1077 (3.1 Å, 3.3 Å) suggesting that while probably not hydrogen-bonding the group shows good electrostatic complementarity to the ATAD2 RVF shelf. In BRD4 BD1, 16 showed a very similar binding mode to 4 (Figure 1e). Surprisingly, even in the presence of the C5'-OMe group, the piperidine ring of 16 adopts a tri-axial conformation. 15 binds to BRD4 BD1 in a similar tri-axial conformation (data not shown). These results illustrate that ligands may adopt higher-energy conformers when their binding site requires.

Table 3 shows further characterization of the potency and selectivity of **16**. By isothermal titration calorimetry a pK_d of 8.1 (K_d 8 nm) was estimated for ATAD2, with no interaction detected with the tandem BRD4 bromodomains (Figure S2, Supporting Information). Whether measured by ITC or TR-FRET, the potency against the BET bromodomains was close to the lower limit, giving a selectivity window of over 2.8 logs. **16** has similar potency in the ATAD2 *Bromosphere* assay, confirming its activity against endogenous full-length ATAD2. The potency against ATAD2 and the closely related ATAD2B bromodomain were comparable by TR-FRET. The

www.angewandte.org

Angewandte

Table 3: Profile of 16 (GSK8814) and its enantiomer 17 (GSK8815). For statistics see Table S1b, Supporting Information.

	16	17
ATAD2 pKd ITC	8.1	5.5
ATAD2 BROMOScan pKi	8.9	n.d. ^[a]
ATAD2 TR-FRET pIC ₅₀	7.3	5.5
ATAD2B TR-FRET pIC ₅₀	7.7	5.5
BRD4 tandem pKd ITC	< 5.3	< 5.3
BRD2 BD1/ BD2 TR-FRET pIC ₅₀	\leq 4.4/ $<$ 4.3	\leq 4.9/ \leq 4.6
BRD3 BD1/ BD2 TR-FRET pIC ₅₀	<4.3/<4.3	\leq 4.6/ \leq 4.7
BRD4 BD1/ BD2 TR-FRET pIC ₅₀	\leq 4.5/ $<$ 3.3	\leq 4.4/ \leq 4.7
BRDT BD1/ BD2 TR-FRET pIC ₅₀	\leq 4.3/ $<$ 4.3	\leq 4.5/ \leq 4.4
CLND solubility [µм]	>439	326

[a] n.d. = not determined.

enantiomeric compound **17** has a similar profile, but is consistently around 2 logs weaker against ATAD2.

The selectivity of **7** and **16** against the wider bromodomain family was assessed in the BROMO*scan* panel (Table S2, Figure S3, Supporting Information). **16** is one of the most selective bromodomain inhibitors so far reported in this panel, with ATAD2 pK_d of 8.9, displaying ≥ 3.0 logs selectivity over 30/33 bromodomains tested, the exceptions being ATAD2B, TAF1 BD2 (2.2 logs) and TAF1L BD2 (2.5 logs). As a further assessment of possible off-target activity, **16** was inactive against an internal GSK panel of 40 targets considered as potential liabilities (data not shown).

With compound **16** showing improved passive permeability over the sulfone analogue **7**, we sought additional evidence for increased cellular permeability. For this, we used a Nano-BRET assay measuring displacement of labelled ATAD2 bromodomain from histone H3.3 (Promega). As expected, treatment with **16** but not **7** or the negative control enantiomer **17** resulted in dose-dependent displacement (Figure 2; Figure S4, Supporting Information). Interestingly, **16** was ineffective at displacing full-length ATAD2 from H3.3.

We attempted to reproduce some of the reported antiproliferative effects of ATAD2 knockdown using **16**. This is the first time that this has been evaluated using a small molecule inhibitor targeting the bromodomain. Disappointingly, effects on colony formation and inhibition of genes involved in cell cycle and division were only seen at high



Figure 2. Unlike the impermeable sulfone analog **7** (grey circles), **16** (black diamonds) displaces the ATAD2 bromodomain construct in a cellular nanoBRET displacement assay. **16** does not displace full-length ATAD2 (crosses). (Error bars = SD).

compound concentrations (Figure S5, Supporting Information). Therefore, we cannot be sure that the effects were caused by ATAD2 inhibition, although they were not seen to such an extent with the negative control 17. Considering the in vitro potency and permeability of 16, these results suggest that the opportunity to regulate proliferation in a therapeutic context via the ATAD2 bromodomain may be limited. There is presently little data associating the anti-proliferative effects seen upon ATAD2 knockdown with its bromodomain, although an intact bromodomain is required for binding acetylated histones.^[18,19] Another group recently concluded that the ATPases of the SMARCA2/4 proteins provide better oncology opportunities than their bromodomains.^[20] The RNA knockdown data may be consistent with reports that other ATAD2 domains are necessary for its function: the Nterminal region is required for oligomerization and binding of acetylated histone H4 and chromatin,^[1,21] and the ATPase for E2-stimulated gene expression.^[18] Perhaps the oligomeric state of ATAD2 results in chromatin binding with such avidity that far more potent bromodomain inhibitors would be needed. However, it is possible that the bromodomain of ATAD2 exerts a more subtle influence and that systems other than those reported here may be more sensitive to inhibitors. For instance, it has recently been proposed that ATAD2 has a more critical function in differentiating embryonic stem cells.^[22] Hence, we hope that reporting this probe, GSK8814 (16) and its less active control GSK8815 (17), to the scientific community will enable others to investigate the biology of ATAD2 further. We recommend not exceeding low-micromolar compound concentrations in cells to avoid any possible unknown off-target effects.

To conclude, we report the optimization of ATAD2 inhibitors for improved cell permeability and selectivity over the BET bromodomains. This was accomplished using what we believe to be a novel use of CF2 as a polar hydrophobic isostere of SO₂. This modification retained the favourable ATAD2 interactions of the sulfone and selectivity over the BETs, yet gave a dramatic improvement in logD and passive permeability. In parallel, an unexpected difference in the bound piperidine conformation in ATAD2 and BRD4 BD1 was exploited to further improve selectivity. The highly unusual tri-axial conformation adopted by 16 in BRD4 BD1 highlights the difference between the conformational preferences of substituted piperidines and cyclohexanes. This effort culminated in the identification of the first reported nanomolar, selective and cell permeable ATAD2 bromodomain inhibitor.

Acknowledgements

We thank Bhumika Karamshi and Laurie Gordon for TR-FRET data, Emma Jones for protein production, Abigail Lucas and Fiona Shilliday for crystallization support, and Jacqui Méndez and Danette Daniels of Promega Corporation for facilitating NanoBRET assays. A.J.B., K.H.C., S.R. and T.K. acknowledge grants from Wellcome Trust (092096), Cancer Research UK (C6946/A14492, RG17001), and BBSRC (RG69031).

www.angewandte.org

These are not the final page numbers!

© 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



Keywords: bioisosteres \cdot conformation analysis \cdot epigenetics \cdot fluorine \cdot medicinal chemistry

- [1] C. Caron, et al., Oncogene 2010, 29, 5171.
- [2] F. Boussouar, M. Jamshidikia, Y. Morozumi, S. Rousseaux, S. Khochbin, *Biochim. Biophys. Acta Gene Regul. Mech.* 2013, 1829, 1010.
- [3] J. X. Zou, L. Guo, A. S. Revenko, C. G. Tepper, A. T. Gemo, H. J. Kung, H. W. Chen, *Cancer Res.* 2009, 69, 3339.
- [4] E. H. Demont, et al., J. Med. Chem. 2015, 58, 5649.
- [5] P. Bamborough, et al., J. Med. Chem. 2015, 58, 6151.
- [6] S. Müller, S. Knapp, *MedChemComm* **2014**, *5*, 288.
- [7] E. P. Gillis, K. J. Eastman, M. D. Hill, D. J. Donnelly, N. A. Meanwell, J. Med. Chem. 2015, 58, 8315.
- [8] J. C. Biffinger, H. W. Kim, S. G. Dimagno, *ChemBioChem* 2004, 5, 622.
- [9] K. Muller, C. Faeh, F. Diederich, Science 2007, 317, 1881.
- [10] B. E. Smart, J. Fluorine Chem. 2001, 109, 3.
- [11] J. A. Howard, V. J. Hoy, D. O'Hagan, G. T. Smith, *Tetrahedron* 1996, 52, 12613.

- [12] L. Brammer, E. A. Bruton, P. Sherwood, Cryst. Growth Des. 2001, 1, 277.
- [13] J. D. Dunitz, ChemBioChem 2004, 5, 614.
- [14] P. Zhou, J. Zou, F. Tian, Z. Shang, J. Chem. Inf. Model. 2009, 49, 2344.
- [15] D. O'Hagan, Chem. Soc. Rev. 2008, 37, 308.
- [16] M. Loudet, F. Metras, J. Petrissans, J. Deschamps, G. Pfister, J. Mol. Struct. 1974, 20, 357.
- [17] O. Tokuda, T. Aikawa, T. Ikemoto, I. Kurinoto, *Tetrahedron Lett.* 2010, *51*, 2832.
- [18] J. X. Zou, A. S. Revenko, L. B. Li, A. T. Gemo, H. W. Chen, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 18067.
- [19] M. Ciro, et al., Cancer Res. 2009, 69, 8491.
- [20] B. Vangamudi, et al., *Cancer Res.* 2015, 75, 3865.
- [21] Y. Zhan, et al., Epigenetics. Chromatin. 2015, 8, 37.
- [22] Y. Morozumi, et al., J. Mol. Cell Biol. 2016, DOI: 10.1093/jmcb/ mjv060.

Received: April 22, 2016 Revised: July 8, 2016 Published online:

© 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Communications



Communications

Epigenetics

Bromodomain

JDCh



ANCCA away: ATAD2 or ANCCA is

a cancer-associated bromodomain-containing protein. Starting from a potent lead, permeability and selectivity over BET bromodomains were improved by 1) using CF_2 as a sulfone bio-isostere to exploit the unique properties of fluorine, and 2) using 1,3-interactions to control the conformation of a piperidine ring. GSK8814 is the first low-nanomolar, selective and cell permeable chemical probe for ATAD2.

6 www.angewandte.org

C 2016 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Angew. Chem. Int. Ed. 2016, 55, 1-6

These are not the final page numbers!