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Article

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Discovery of epigenetic regulator I-BET762: lead optimization to afford a clinical candidate inhibitor of the BET bromodomains

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ABSTRACT. The bromo and extra C-terminal domain (BET) family of bromodomains are involved in binding epigenetic marks on histone proteins, more specifically acetylated lysine residues. This paper describes the discovery and structure-activity relationship (SAR) of potent benzodiazepine inhibitors that disrupt the function of the BET family of bromodomains (BRD2, BRD3 and BRD4). This work has yielded a potent, selective compound I-BET762 that is now under evaluation in a phase I/II clinical trial for nuclear protein in testis (NUT) midline carcinoma and other cancers.

Introduction

The bromodomain and extra-terminal domain (BET) family of proteins (BRD2, BRD3, BRD4 and BRDT) activate transcription through their ability to recognize specific ε-*N*-acetyl-modified lysine residues found within histone tails and other proteins.[1] While three members of this family (BRD2, BRD3 and BRD4) are ubiquitously expressed, the fourth member, BRDT, is a tissue-restricted, chromatin-associated protein expressed in pachytene spermatocytes, diplotene spermatocytes, and round spermatids.[2] BRD2-4 function as chromatin scaffolds that recruit Polymerase II (RNA Pol II) to the PAFc complexes, and in the case of BRD4 P-TEFb,[3] thus ensuring RNA Pol II-dependent transcriptional initiation and elongation respectively.

The recent disclosure of potent, selective small molecule inhibitors of the BET family of bromodomains demonstrate that epigenetic reader proteins may be as tractable to small molecule drug discovery as their epigenetic enzyme counterparts.[4] To date, a small number of structurally diverse chemotypes have been reported to target the acetyl-binding pocket of the BRD domains of the BET proteins and demonstrate a broad spectrum of desirable biological effects (Chart 1).

Of these, I-BET762[5] **1** has recently entered clinical trials for NUT midline carcinoma (NMC).[6] NMC is a rare, lethal and aggressive tumor with a median overall survival of 6.7 months,[7] in which a NUT gene translocation generates a fusion protein with BRD4 or BRD3 proteins that is retained strictly in the cell nucleus via interactions with chromatin. Functional studies in patient-derived NMC cell lines have validated the essential role of the BRD4–NUT fusion oncoprotein in maintaining the characteristic proliferation advantage and differentiation block of this malignancy which is arrested following treatment with the thienodiazepine BET inhibitor JQ1 (compound **2**).[8] JQ1 is also active against myeloma,[9] lymphoma,[10] acute lymphoblastic leukemia[11] and neuroblastoma[12] *in vitro* and *in vivo*, while a second class of quinoline based BET protein inhibitors, exemplified by I-BET151 (**3**), was shown to have considerable pre-clinical activity against acute leukemia including MLL-related acute myeloid leukemia.[13] More recently, publications from Pfizer/SGC[14] and the Chinese Academy of Sciences[15] described 3-methyl-1,2,3,4-tetrahydroquinazolin-2-ones **4** and 2-thiazolidinones **5** respectively, as new chemical probes for BET protein inhibition.

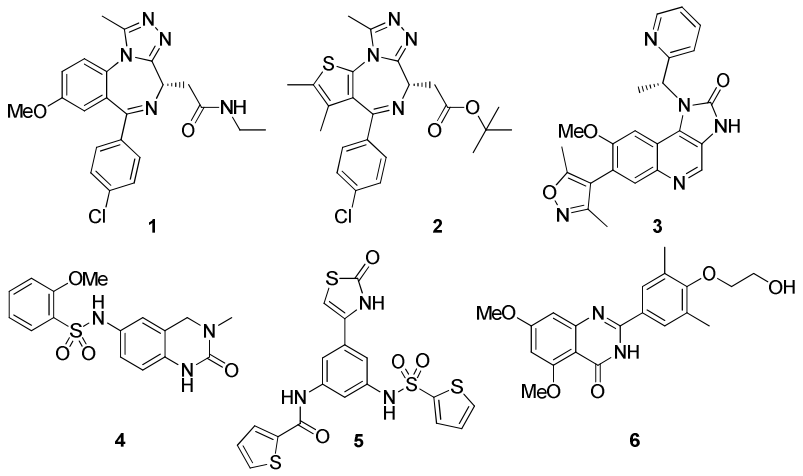


Chart 1. Reported structurally diverse BET inhibitors.

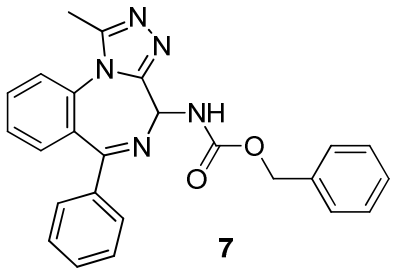
We recently reported the discovery of compounds using a reporter assay for ApoA1 upregulation, whose molecular targets were subsequently elucidated to be the BET family of bromodomain-containing proteins.[16] A recent disclosure from the Canadian-based biotechnology company Resverlogix has reported the mechanism of action for its Phase IIb asset RVX-208 **6** (Chart 1),[4c] which was developed as a regulator of ApoA1 gene transcription, to be also through inhibition of the BET bromodomain modules. This provides increased confidence in the clinical tolerability of these novel agents and highlights the value of understanding the SAR around inhibitors of this protein family to fully exploit and test the therapeutic potential of this target class.

In this manuscript we describe the medicinal chemistry program that led to the discovery of the potent and selective small molecule I-BET762 or GSK525762 which is currently under clinical development.

RESULTS AND DISCUSSIONS

Discovery of small molecule upregulators of ApoA1

Efforts to target endogenous upregulation of ApoA1 expression are hampered by the absence of an obvious molecular mechanism around which to build a drug discovery effort. In 2001, we therefore generated a stable human HepG2 hepatocyte cell-line containing an ApoA1 luciferase reporter and used this to screen compounds in order to identify molecules with the ability to upregulate reporter gene activity. Diversity and targeted screening approaches led to the identification of the benzodiazepine (BZD) **7** (Figure 1) which showed potent induction of the ApoA1 reporter gene with an EC_{170} of 0.22 μM.[17]



7

Apo A-1 Luc EC_{170} = 0.22 μM
Brd2/3/4 pIC_{50} 5.9/6.2/6.3

Figure 1. Benzodiazepine hit **7**.

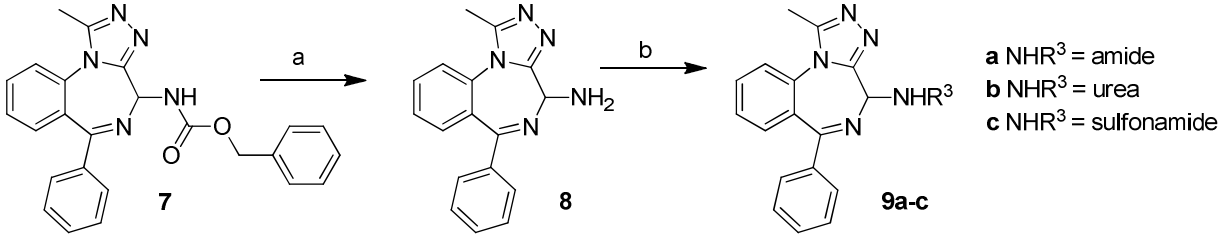
Compound **7** was considered a suitable starting point for lead optimization. In the absence of any knowledge of the molecular target at this time, a program of medicinal chemistry was carried out to optimize the potency against the ApoA1 upregulation assay.[16] Having subsequently identified the targets of our compounds as the bromo and extra-terminal (BET) family of bromodomains,

we will describe their affinities towards all BRD subtypes and the lead optimization program that led to the discovery of the clinical compound I-BET762 (**1**).

Chemistry

The 1,4-benzodiazepine skeleton is one of medicinal chemistry's most widely used scaffolds. First discovered in the 1960s,[18] there are now many marketed pharmaceutical agents (e.g. Bromozepam) that contain a 1,4-benzodiazepine motif principally as GABA receptor positive allosteric modulators for CNS clinical indications, and a number of efficient synthetic routes to 3-amino-1,4-benzodiazepines have been described in the literature. The design of novel ApoA1 upregulators was performed to explore all different chemical modifications around the BZD scaffold as depicted in Schemes 1 to 7. 3-Amino-1,4-benzodiazepine derivatives **7**, **8** and **9a-c** described in this manuscript were prepared using the synthetic route described in Scheme 1.[19, 20] Commercially available benzodiazepinone **7**[21] was first submitted to catalytic hydrogenation and the Cbz group was removed leading to amine **8** as a key intermediate to explore diversity at position R^3. Since compound **7** tolerates a polar carbamate linker, we explored other spacers such as amide **9a**, urea **9b**, sulfonamide **9c** using standard chemistries.

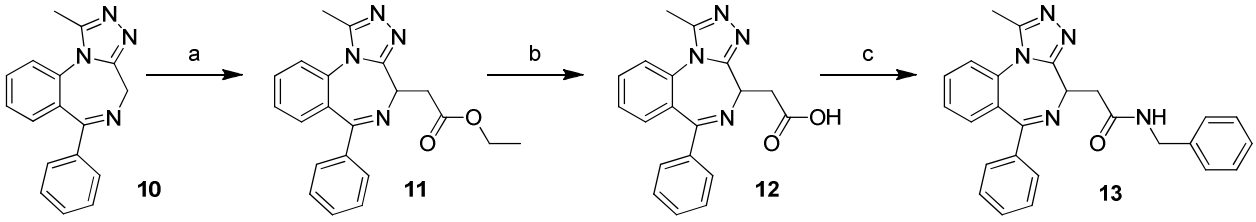
Scheme 1. Synthesis of substituted 3-amino-1,4-benzodiazepine derivatives **7**, **8** and **9a-c**[a]



[a]Reagents and Conditions: (a) 1,4-cyclohexadiene, cat. Pd/C, MeOH, RT, 98%; (b) R^3 introduction, see Experimental Section, 2 – 70%.

As depicted in Scheme 2, BZD **13** bearing an acetamide linker was obtained starting from known alprazolam analogue **10**.[22] Deprotonation of the BZD methylene with LiHMDS in THF at low temperature followed by addition of ethyl bromoacetate led to alkylated product **11** in modest yield. The latter was saponified using sodium hydroxide giving acid **12** in good yield. Finally, standard peptide coupling conditions with HATU as coupling reagent afforded BZD acetamide **13** in 71% yield.

Scheme 2. Synthesis of benzodiazepine **13**[a]

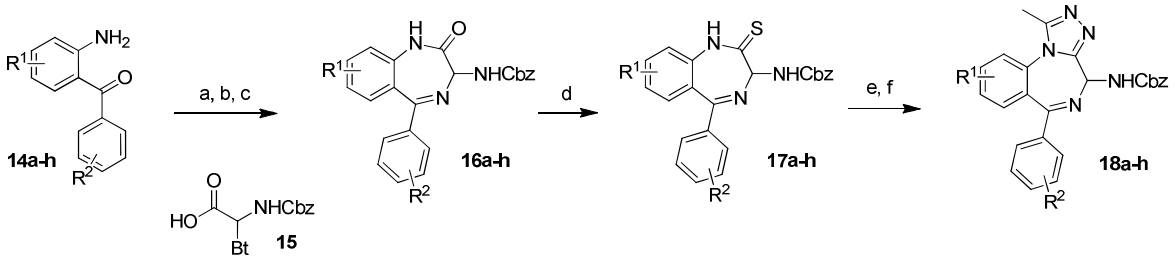


[a]Reagents and Conditions: (a) Ethyl bromoacetate, LiHMDS, THF, -78°C to RT, 48%; (b) 1N NaOH, EtOH/THF, RT, 78%; (c) Benzylamine, HATU, Et_3N, DMF, RT, 71%.

As depicted in Scheme 3, 3-amino-1,4-benzodiazepines **16a-h** were synthesized with good overall yields using a reported sequence[31] starting from aminobenzophenones **14a-h** and

Katritzky reagent **15**.[23] Thionation with Lawesson's reagent afforded thioamides **17a-h** and triazole ring construction using conditions previously described[21] led to test compounds **18a-h**.

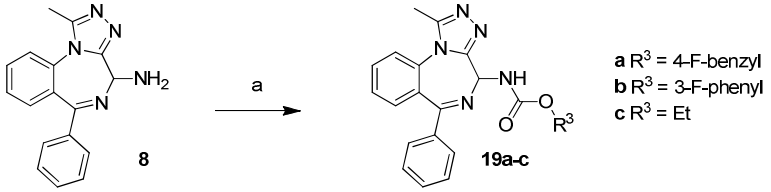
Scheme 3. Representative synthesis of substituted 3-amino-1,4-benzodiazepine derivatives[a]



[a]Reagents and Conditions: (a) **15**, EDCI, cat. DMAP, DCM, 0°C; (b) NH_3, MeOH, RT; (c) NH_4OAc, AcOH, RT; (d) Lawesson's reagent, toluene, reflux; (e) $H_2N\text{-}NH_2 \bullet H_2O$, MeOH, RT; (f) $MeC(OMe)_3$, PPTS, MeOH, reflux; **Bt** = benzotriazole.

Exploration of the carbamate substitution was performed using the chemistry shown in Scheme 4. Intermediate **8** was reacted with a variety of formate derivatives to afford the desired carbamates **19a-c**.

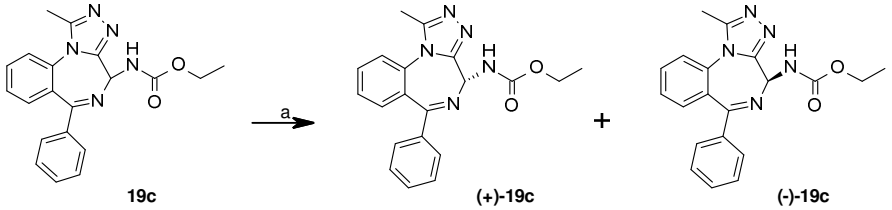
Scheme 4. Synthesis of substituted 3-amino-1,4-benzodiazepine derivatives **19a-c**[a]



[a]Reagents and Conditions: (a) R^3 introduction, see Experimental Section, 10 – 62%.

Enantiomers of compound **19c** were separated by chiral preparative HPLC using a Chiralpak AD column and an isocratic mixture of EtOH/heptane: 80/20 (Scheme 5). The absolute stereochemistries of **(+)-19c** and **(-)-19c** were assigned using Vibrational Circular Dichroism (see Supplementary Information Section 2.2 for details) and found to be *R* and *S* respectively.

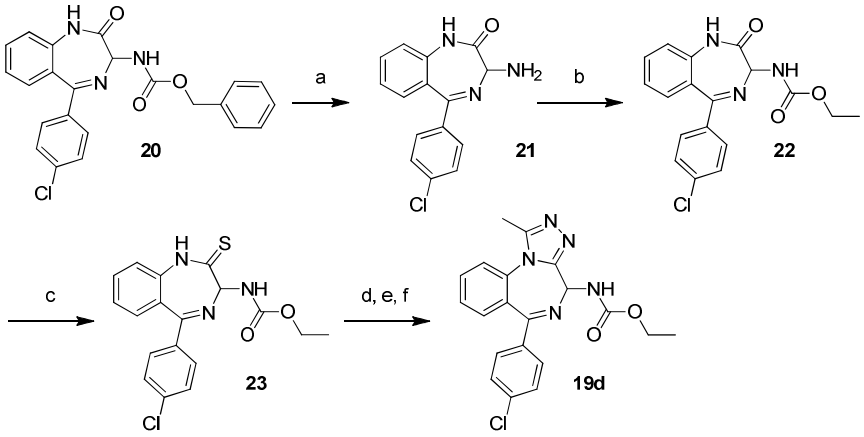
Scheme 5. Chiral separation of compound **19c**[a]



[a]Reagents and Conditions: (a) chiral preparative HPLC (Chiralpak AD column, EtOH/heptane : 80/20).

Alternatively, compound **19d** was prepared using chemistry depicted in Scheme 6. Benzodiazepinone **20**[24] was subjected to acid hydrolysis to give amine **21** in good yield. Upon reaction with ethyl chloroformate in the presence of triethylamine, compound **22** was obtained from **21**. Thioamide **23** was prepared using the same chemistry described earlier and, finally, formation of the triazole ring was performed in three steps involving addition of hydrazine followed by a quench with acetylchloride. Cyclization occurred under refluxing acid conditions giving test compound **19d** in good yield over the three steps.

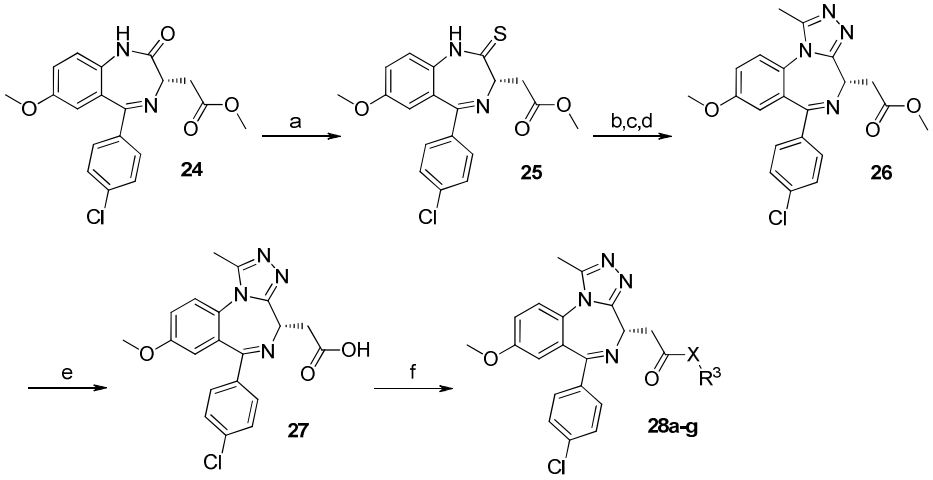
Scheme 6. Synthesis of substituted 3-amino-1,4-benzodiazepine derivatives **19d**[a]



[a]Reagents and Conditions: (a) 37% HBr, AcOH, 80°C, 92%; (b) ethyl chloroformate, Et_3N, THF, 0°C to RT, 83%; (c) Lawesson's reagent, toluene, reflux; (d) $H_2N\text{-}NH_2\bullet H_2O$, MeOH, RT; (e) AcCl, DIPEA, THF, 0°C; (f) AcOH, reflux, 55% over 3 steps.

Acetamide BZD derivatives were prepared according to Scheme 7. As described in our previous report,[16] the thionation step of the synthetic route to racemic final compounds involved the use of Lawesson's reagent. Under those conditions, thioamide **25** was obtained as a racemic mixture. In order to obtain the final compounds as active pure (*S*)-enantiomers, we separated enantiomers by preparative chiral HPLC and tested them to identify the active samples, which proved to be arduous. We thus investigated the thionation step and found that using phosphorus pentasulfide in the presence of sodium carbonate led to the formation of the desired product without epimerization. The completion of the synthesis was consistent with that described earlier and afforded compounds **26, 27** and **28a-g**.[25] Compounds **29** and **30** were prepared using the same synthetic pathway.

Scheme 7. Synthesis of BZD acetamide derivatives **26, 27** and **28a-g**[a]



[a]Reagents and Conditions: (a) P_4S_{10}, Na_2CO_3, 1,2-DCE, 65°C; (b) H_2N-NH_2, THF, 5°C to 15°C; (c) AcCl, THF, 0°C; (d) AcOH, THF, RT; (e) NaOH, THF, RT; (f) R^3XH, coupling reagent, base, solvent (X= NH or O).

Biological activities

We earlier reported[16] that ApoA1 upregulation in HepG2 cells tracks *in vitro* binding to the three subtypes of BET proteins extremely well. Although lead optimization was carried out against ApoA1 upregulation as the primary assay, in this manuscript for clarity we will discuss only the BET affinities of the benzodiazepine compounds. ApoA1 upregulation data for all compounds is fully detailed in the Supporting Information section (Tables S1 to S4).

Compound **7** was found to be around micro molar IC_{50} in our fluorescence polarization assay (Figure 1) against each of the three BET isoforms. This is more potent than simpler benzodiazepine analogues lacking the benzyl carbamate group, such as Alprazolam[26] or the analogous thienotriazolodiazepine GABA receptor positive allosteric modulator Etizolam[36] (Figure 2A). The crystal structures of these simple unsubstituted thienotriazolodiazepines in complex with the *N*-terminal bromodomain of BRD4 were solved and found to adopt a binding mode perfectly superimposable with that of **7** previously described.[16] In these structures, the

triazolyl ring forms close hydrogen-bonding interactions with the side chain NH of Asn140 and a network of water molecules (Figure 2). The BZD fused phenyl ring fills a narrow lipophilic channel between Leu92 and Pro82, termed the "ZA channel", and the pendant phenyl ring packs against Ile146, on the "WPF shelf".[16]

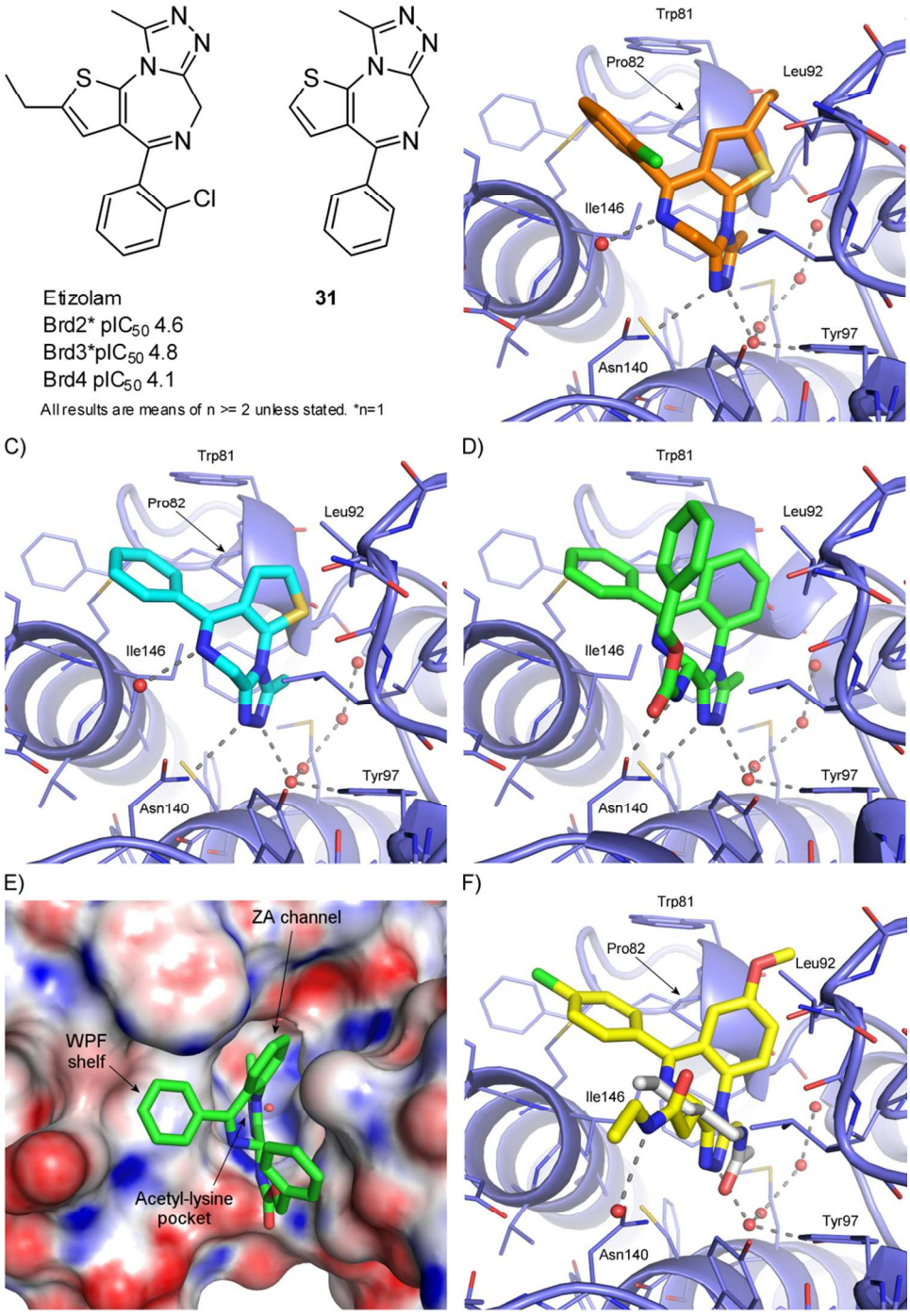


Figure 2: A) GABA receptor positive allosteric modulator Etizolam and its analogue **31**. Crystal structures of BRD4 in complex with B) Etizolam, C) **31**, D) **7** (the benzyl carbamate substituent depicted has good density within this structure but places the benzyl group in an

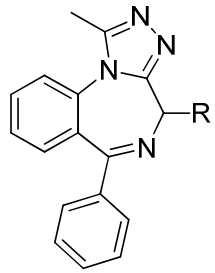
orientation distinct from that seen in BRD2 suggesting this group may be mobile), E) **7** showing the electrostatic potential surface of BRD4, F) Comparative binding to BRD4 of compound **1** (yellow carbons) and the acetyl-lysine sidechain of a histone H4 peptide (white carbons, PDB entry 2yel).[4c]

In its complex with BRD4, the additional carbamate group of **7** extends towards the volume that would be occupied by the *N*-terminal side of the acetyl-lysine peptide chain. The carbamate NH makes hydrogen-bonding interactions to the side-chain of Asn140, however no further specific interactions are observed for the rest of the carbamate. The carbamate benzyl group is found to adopt distinct conformations in BRD2 and BRD4, again suggestive that this group does not make strong interactions with the protein and are mobile. A similar conclusion was reached by Filippakopoulos *et al.* in their studies comparing Alprazolam and Midazolam to (+)-JQ1.[26]

We decided to explore alternative functionalities to the carbamate (Table 1) capable of maintaining the interaction of the NH with Asn140. We removed it completely (compound **8**, Table 1) or replaced it with an amide (compound **9a**), an urea (compound **9b**) and a sulfonamide (compound **9c)**. We also investigated linkers where the NH group is moved further from the benzodiazepine core, for example the acetamide compound **13**. Urea **9b** displays similar binding affinities to **7**, but amide **9a** and sulfonamide **9c** were less active. We attribute this to steric non-complementarity between the bromodomain site and benzyl substituents when attached via linkers whose preferred geometry is different to the linear carbamate and urea. This hypothesis has been confirmed by X-ray crystallography (data not shown).

Compounds were also tested for their anti-inflammatory properties assessing their ability to inhibit IL-6 production after a LPS-challenge in peripheral blood mononuclear cells (PBMC). In this cellular assay, compound **9b** was found to be much weaker than **7,** probably due to lower solubility of the urea compared to carbamate. Overall, the carbamate substituent looked the most attractive on the benzodiazepine ring 3-position.

Table 1. SAR around the BZD ring RHS substituent.[a]



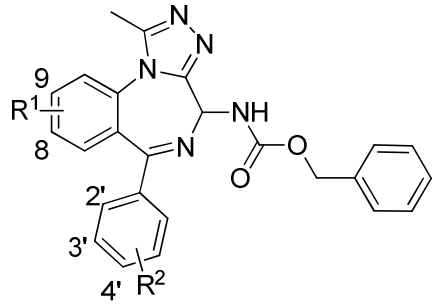
Cmpd	R	BRD2 (FP) pIC_{50}	BRD3 (FP) pIC_{50}	BRD4 (FP) pIC_{50}	PBMC (IL-6) pIC_{50}
7	$NHCO_2Bn$	5.9	6.2	6.3	6.8
8	NH_2	4.6[c]	4.9[c]	4.7[c]	NT[b]
9a	$NHCOCH_2CH_2Ph$	4.8	5.5	5.4	NT[b]
9b	$NHCONHCH_2Ph$	5.7	6.1	5.9	6.4
9c	$NHSO_2CH_2Ph$	4.5	5.2	5.2	4.8
13	$CH_2CONHCH_2Ph$	5.6[c, d]	5.7	5.2	5.3

[a]All results are means of n ≥ 2 unless stated. [b]NT = Not Tested. [c]n = 1. [d]Following repeat testing, activity was observed but no pIC_{50} was defined on six separate test occasions due to low max response observed (<50% inhibition) at the highest concentration tested.

We next investigated the substituents on the fused phenyl ring of the benzodiazepine (Table 2). This aromatic ring binds into the lipophilic ZA channel of the bromodomain (Figure 2). Introduction of electron-withdrawing (compounds **18a** and **18b**) or donating groups (compounds **18c** and **18d**) at either position 8 or 9 did not have much effect on affinities toward the 3 subunits except for the 8-nitro derivative **18a** which lost quite some potency on the bromodomain and in the cell assay. The other substituents were well tolerated and the 8-methoxy compound **18d** was found to be the best, especially taking into account its potency within the cell assay.

We then turned our attention toward the pendant phenyl ring that interacts with the lipophilic WPF shelf (Figure 2) and first tested all positions of the ring. Introduction of a methoxy at the *ortho* position of the ring abolished BRD affinity, as exemplified by compound **18e**. This can be rationalised with reference to the X-ray structure of Etizolam (Figure 2B). The ortho-chloro substituent induces a rotation in the inter-ring torsion angle, leading to a decrease in the surface area of the pendant phenyl ring in contact with the hydrophobic surface of Ile146 on the WPF shelf. When the methoxy substituent was introduced at either *meta* or *para* position, compounds **18f** and **18g** respectively were found equally potent against the 3 BRD subunits and in the cell assay, probably due to the fact that those two positions point toward the solvent and would not make direct interactions with the bromodomain. The *para* position of the pendant phenyl ring tolerates most substitutions. For example, a methoxy group (compound **18g**) could be replaced by a methyl (compound **18h**) without affecting potency.

Table 2. SAR around both phenyl rings of the BZD.[a]



Cmpd	R^1, R^2	BRD2 (FP) pIC_{50}	BRD3 (FP) pIC_{50}	BRD4 (FP) pIC_{50}	PBMC (IL-6) pIC_{50}	Central BZDR pIC_{50}
7	H, H	5.9	6.2	6.3	6.8	6.2
18a	8-NO_2, H	4.9	5.5	5.5	5.7	NT[b]
18b	8-Cl, H	5.5	5.8	5.9	6.2	7.3[c]
18c	9-Me, H	5.6[c]	5.9[c]	5.8[c]	6.2	6.1[c]
18d	8-OMe, H	6.1	6.1	6.3	6.9	NT[b]
18e	H, 2'-OMe	5.0	5.3	5.3	5.0	NT[b]
18f	H, 3'-OMe	5.5	6.0	6.0	6.3	<5.0[c]
18g	H, 4'-OMe	5.7	6.0	5.9	6.5	<5.0[c]
18h	H, 4'-Me	5.8	6.0	5.9	NT[b]	<5.0[c]

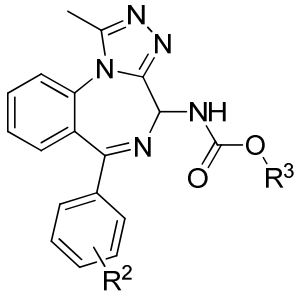
[a]All results are means of n ≥ 2 unless stated. [b]NT = Not Tested. [c]n = 1.

As discussed earlier, the 1,4-benzodiazepine motif has been used principally as GABA receptor positive allosteric modulators for CNS clinical indications. From the start of this work, we recognized the structural similarity between our benzodiazepine ApoA1 upregulators and alprazolam[26], and thus tested the ability of our compounds to inhibit diazepam binding to the central GABA receptor. Compounds **7**, **18b** and **18c** were found active in this assay with a pIC_{50}

of 7.3 for **18b**. The hypothesis developed by Filippakopoulos *et al.* that JQ1 has no significant GABA receptor activity due to its bulky substitution at the 3 position of the benzodiazepine ring system[26] is inconsistent with our observations, since all our compounds share a similar bulky group but are still active on the central GABA receptor. Nonetheless, when a substituent was introduced at either *meta* or *para* position of the pendant phenyl ring, compounds **18f, 18g** and **18h** were found to be inactive on the GABA receptor, thereby giving us an opportunity to solve this selectivity issue.

Having defined that the carbamate function was the best linker at position 3 of the benzodiazepine ring, we turned our attention to optimizing this substituent. From the crystal structure of **7** in BRD4, this group points toward the bulk solvent, and provides limited potential to improve bromodomain affinity. However, optimization of this part of the molecule allowed us to manipulate the physicochemical properties of the compounds (Table 3). Introduction of a fluorine atom at the *para* position of the benzyl group (compound **19a**) maintained BRD affinities and cellular activity. Our attempt to truncate the benzyl group by a 3-fluorophenyl (compound **19b**) resulted in reduced affinities toward all three bromodomains. We then tried to reduce both molecular weight (MW) and lipophilicity (clogP) of the molecule to a range more desirable for oral drugs (MW <400, clogP <3) by replacing the benzyl group by an alkyl such as in compound **19c**. This had a similar affinity to **7** against both BRD3 and BRD4 although there was a slight drop in cellular potency. We then investigated the contribution of each enantiomer to the BRD affinity of compound **19c** and showed that only enantiomer **(+)-19c** was found active. Ultimately this proved to be of absolute (*R*)-configuration, whereas its enantiomer **(-)-19c** had no significant activity.

Table 3. SAR around the BZD carbamate substituent.[a]



Cmpd	R^2, R^3	BRD2 (FP) pIC_{50}	BRD3 (FP) pIC_{50}	BRD4 (FP) pIC_{50}	PBMC (IL-6) pIC_{50}	MW	clogP[c]	$t_{1/2}$[d] h
7	H, Bn	5.9	6.2	6.3	6.8	423	2.4	0.58
19a	H, 4F-Bn	5.7	6	6.3	6.5	441	2.5	0.33
19b	H, 3F-Ph	5.5[e]	5.9[e]	5.8[e]	NT[b]	427	3.1	NT[b]
19c	H, Et	5.6	6	6.1	6.3	361	2.1	0.56
(-)-19c	H, Et	4	4	4	NT[b]	361	2.1	NT[b]
(+)-19c	H, Et	5.9	6.3	6.4	6.7	361	2.1	0.23
19d	4'-Cl, Et	5.6	5.9	6.1	NT[b]	396	2.8	0.75

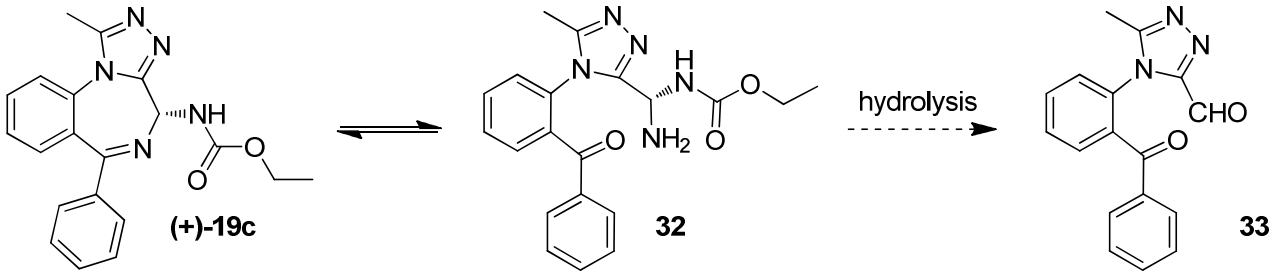
[a]All results are means of n ≥ 2 unless stated. [b]NT = Not Tested. [c]calculated logP (Biobyte/Daylight). [d]$t_{1/2}$ determined in pH 2 buffer by HPLC. [e]n = 1.

As discussed earlier, we speculated that compounds unsubstituted on the pendant phenyl ring such as **19a-c** would be active on the GABA receptor. In order to eliminate this undesired activity, we introduced a substituent in the *para* position of the pendant ring. Compound **19d** was equipotent to **19c** and **7** on the BRD subtypes but completely inactive when tested in the central

GABA receptor binding assay ($pIC_{50} < 5$), thus reinforcing our belief that this pendant ring substitution was key to GABA receptor selectivity of the benzodiazepine compounds.

When discussing the progression of such compounds, we speculated that the presence of two nitrogens on the benzodiazepine 4-carbon could lead to instability of the compounds. It has been shown that triazolobenzo- and triazolothienodiazepines can undergo a ring-opening reaction in an acidic aqueous solution,[27] leading to a postulated intermediate **32** in the case of compound (+)-**19c** as shown in Scheme 8. The latter could easily be hydrolyzed in acidic conditions to form the benzophenone **33**. This instability at low pH could lead to a rapid degradation of **(+)-19c** which was incompatible with our target of achieving good oral exposure. We tried to discharge this concern by setting up an assay where compound half-life could be measured in an acidic solution that mimicks gastric pH. Compounds were suspended in a pH 2.0 buffer and their half-life ($t_{1/2}$) measured. All compounds tested from this series were found to undergo rapid ring opening under these low pH conditions ($t_{1/2} < 1$ h, Table 3), with the active enantiomer **(+)-19c** being the most rapidly converted. Since this may be a possible route for active metabolite formation, we turned our attention to structural changes that could mitigate this potential risk.

Scheme 8. Postulated mechanism for a potential low pH degradation pathway



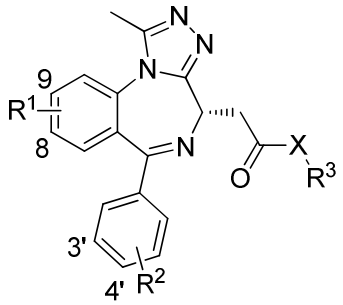
Additionally, we also wanted to address anticipated issues in achieving an efficient asymmetric synthesis for compounds of this series. Although there is one report that describes the synthesis of an enantiomerically pure aminobenzodiazepine using a crystallization-induced asymmetric transformation,[28] most reported syntheses involve a chiral resolution step.[29] We tried to solve those two concerns together by designing a compound that would be acid-stable and amenable to an enantioselective synthesis. One way of improving acid stability would be to remove one nitrogen atom at position 3 of the benzodiazepine ring. That would lead to compounds like the benzodiazepine acetamide **13** discussed earlier, which was found to be acid stable under the same conditions ($t_{1/2}$ > 2h). We also recognized that this compound could be synthesized from natural aspartic acid, and was amenable to an enantioselective synthesis as shown in Scheme 7. Compound **13** showed only modest affinity toward the BRD subunits (Table 1) but we speculated that applying SAR learnt during the optimization of the aminobenzodiazepine series would ultimately lead to improvements. We also knew that compound **13** had no affinity toward the central BZD receptor (pIC_{50} < 5.0) which reinforced our interest in this sub-series. The inactivity on the GABA receptor was true for all compounds tested in this sub-series (see Supporting Information table S4 for compounds tested).

In order to improve BRD affinity, we investigated the structure-activity relationships on the amide linker exemplified by **13**. The best features from our earlier compounds were

incorporated, specifically the methoxy group at the 8 position of the benzodiazepine exemplified in compound **18d** (see Table 2), and a *para* chlorine atom on the pendant phenyl ring, to yield compounds shown in Table 4. As seen in the aminobenzodiazepine series, a variety of substituents were tolerated in this part of the molecule, probably due to the fact that this group is solvent exposed. Substituted phenyl (compound **28a**), heteroaromatic ring (compounds **28b-d**) and alkyl substituted analogues (compounds **28e** and **1**) were found equipotent toward the three BRD tested. This position was therefore used to improve the overall physicochemical properties of the compounds. Aromatic substituents proved to be more lipophilic than desirable (clogP >3) although this made little impact on the solubility, which was good for all compounds. Alkyl-substituted analogues (e.g. **28e** and **1**) offered a better profile regarding lipophilicity, with **1** being one of the most potent acetamide analogues in the cell assay. We then assessed the importance of the methoxy at position 8 and tested analogue **29** having the methoxy at position 9 of the fused ring of the BZD. This compound is less potent in both BRD binding and cellular assays, reinforcing the preference for substitution at position 8. When we explored the *meta* position of the pendant phenyl ring, compound **30** bearing a fluorine atom was found less active than **1**. Carboxylic acid **27** was also tested and found to be barely active, a result which could be explained by the likely proximity of the acid to the side-chain carbonyl oxygen of Asn140 (Figure 2D). Methyl ester **26** and butyl ester **28g** had excellent affinity toward the three BRD sub-types and a very good potency in the cell assay, probably due to a concordant increase in their lipophilicity. Ester **28g** was the most potent compound we identified in the cell assay, with a pIC_{50} of 7.4. Consistent with other reported BET inhibitors there is no selectivity observed with

these molecules between BET protein isoforms (Table S4), reflecting the high degree of sequence homology between the acetyl lysine binding sites of these proteins.[16a]

Table 4. SAR around the BZD acetamide template.[a]



Cmpd	R^1	R^2	X	R^3	BRD4 (FP) pIC_{50}	PBMC (IL-6) pIC_{50}	Solubility in FaSSIF µg/mL	clogP[d]
13	H	H	NH	Bn	5.2	5.3	1	2.9
28a	8-OMe	4'-Cl	NH	4F-Ph	6.3	5.7	105	4.2
28b	8-OMe	4'-Cl	NH	2-thiazole	6.4	6.0	100	3.3
28c	8-OMe	4'-Cl	NH	2-pyridyl	6.4	6.5	115	3.1
28d	8-OMe	4'-Cl	NH	3-pyridyl	6.3	NT[b]	95	3.1
28e	8-OMe	4'-Cl	NH	*c*-propylmethyl	6.3	NT[b]	111	2.8
1	8-OMe	4'-Cl	NH	ethyl	6.2	6.5	122	2.4
29	9-OMe	4'-Cl	NH	ethyl	5.7[c]	6.2	NT[b]	2.4
30	8-OMe	3'-F	NH	ethyl	5.9	NT[b]	116	1.8
27	8-OMe	4'-Cl	O	H	5.1[c]	<4	113	2.4
26	8-OMe	4'-Cl	O	methyl	6.6	6.9	115	2.8

28g	8-OMe	4'-Cl	O	butyl	6.4	7.4	104	4.4

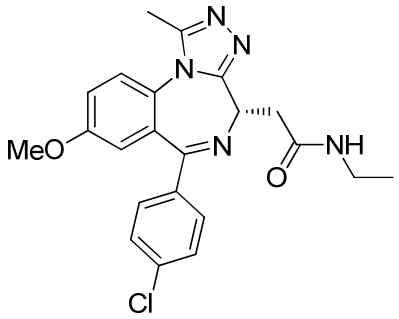
[a]All results are means of n ≥ 2 unless stated. [b]NT = Not Tested. [c]n = 1. [d]calculated logP (Biobyte/Daylight).

From this set of compounds, due to its good overall balance of properties we decided to select compound **1**, also known as I-BET or I-BET762, for further profiling. The X-ray structure of **1** bound to BRD4 (Figure 2F) illustrates an unexpected replacement of the direct hydrogen bond from the sidechain NH of Asn140 to the carbamate NH of **7** by a water-bridged interaction through the amide NH of **1**. Other binding features of the two complexes are very similar.

I-BET762 Preclinical Developability Profile

I-BET762 has high *in vitro* passive permeability (167 nm/sec) with similar moderate free fractions across all the species investigated (f_{ub} ca. 0.2; Table 5). I-BET762 has high solubility in physiologically relevant media demonstrating solubility > 3 mg/mL in all vehicles tested (Simulated Gastric Fluid (pH 1.6) > 4 mg/mL, fasted state simulated intestinal media (pH 6.5) > 3 mg/mL and fed state simulated intestinal fluid (pH 6.5) > 5 mg/mL*). In vitro* data suggest that I-BET762 has a low potential to inhibit the major human CYP isoforms (1A2, 2C9, 2C19, 2D6, 3A4 IC_{50}'s ≥ 33 μM) with no evidence for time dependent inhibition of CYP2D6 or CYP3A4. *In vitro* clearances determined with liver microsomes and hepatocytes were low across all species, including human (CL_i ≤ 1.7 mL/min/g liver, all systems).

Table 5. Summary of *in vitro* DMPK data for **1**



Assay	Test system	Mouse	Rat	Dog	Primate[b]	Human
Free Fraction (f_{ub})[a]	Blood	0.21	0.18	0.24	NT[c]	0.19
Metabolic stability	Microsomes	<0.53	1.45	0.69	0.20	<0.53
CL_i, (mL/min/g liver)	Hepatocytes	<0.86	<0.86	< 1.7	<0.085	<0.86

[a]Measured using rapid equilibrium dialysis at a nominal concentration of 1000 ng/mL. [b]Cynomolgus monkey. [c]NT = Not Tested.

The pharmacokinetic properties of I-BET762 were then investigated in 4 preclinical species: mouse, rat, dog and primate (Table 6). I-BET762 has a low blood clearance in dog and primate (≤ 30% liver blood flow in both species) and a moderate blood clearance in the mouse and rat (≤ 70% liver blood flow in both species). The volumes of distribution were moderate (ca. 1.8 L/kg) in rat, dog and primate, but high in mouse (ca. 6.5 L/kg) indicating distribution into the tissues. The terminal half-life was short in rat (ca. 0.5 h) and longer in mouse, dog and primate (ca. 1.5 - 5.9 h, respectively). Oral bioavailability of I-BET762 was moderate (ca. 27%) in the rat and higher in mouse, dog and primate (44 – 61%).

The solubility and permeability data suggested that I-BET762 should diffuse well across membranes. However, *in vitro* metabolic stability data for I-BET762 were found not to predict the *in vivo* PK profile across the four pre-clinical species. The favourable pharmacokinetic

profile observed in the mouse, dog and primate was not observed in the rat despite broadly similar *in vitro* clearance values. The exact reasons for this are not understood, but it is known that the rat often poorly predicts oral bioavailability in human.[32] Using all the available data, human pharmacokinetic predictions were performed using several techniques including allometric scaling and physiologically based pharmacokinetic modelling (PBPK) using Gastroplus™ (version 5.0; Simulations Plus Inc., Lancaster, CA, USA; data not shown). Despite the less than optimal pharmacokinetic profile observed in the rat a favourable human pharmacokinetic profile was predicted thus justifying clinical progression.

Table 6. Mouse**,** rat, dog and primate pharmacokinetic parameters for **1**[a]

Species	$AUC_{0\text{-}inf}$ ng.h/mL	CL_b mL/min/kg	V_d L/kg	$T_{1/2}$, i.v. h	F, p.o. %
Balb/c Mouse	468	75	6.5	1.5	61
CD Rat	201	63	1.8	0.5	27
Beagle Dog	4741	5	1.8	5.9	44
Primate[b]	3338	14	1.5	1.5	52

[a] Values are mean, n=3. IV dose (1 mg/kg) was 1 h infusion in DMSO (2%, v/v) Kleptose HPB (10%, w/v) in saline (0.9%, w/v). PO dose (3 mg/kg) suspension in 1% (w/v) methylcellulose (400 cps) (aq). [b] Cynomolgus monkey. Values are mean, n=4. IV dose (2 mg/kg) was 1 h infusion in DMSO (4%, v/v) HPB (20%, w/v) in water. PO dose (5 mg/kg) suspension in 0.5% (w/v) HPMC/ TWEEN80 1% in water.

I-BET762 was tested in a non-GLP bacterial mutation screening assay (Ames test) with *Salmonella typhimurium* TA1535, TA1537, TA98, TA100 and *Escherichia coli* WP2uvrA(pKM101) in the presence and absence of rat S9-mix. I-BET762 is not mutagenic

when tested in either the presence or absence of S9-mix. The compound had no appreciable activity ($pIC_{50}/pEC_{50} \leq 4.8$) in an in-house developability panel containing 38 assays related to safety[33] or an external panel containing 50 selectivity assays[34] (<30% inhibition at 10 μM). Moreover, representative bromodomain containing proteins BAZ2B, SP140, ATAD2, CREBBP and PCAF were shown not to interact with I-BET.[7]

In vivo studies using I-BET762 have demonstrated efficacy in a range of oncology and immunoinflammatory models.[5, 9, 10] Consistent with previously reported effects of I-BET on T-cell function *in vitro*,[35] I-BET dose-dependently inhibited ear swelling in a rat model of delayed-type hypersensitivity (DTH) at a comparable level to the positive control rapamycin (Figure 3). These data provide further evidence of I-BET efficacy in an *in vivo* setting, highlighting further potential clinical utility of BET inhibitors beyond the oncology arena.

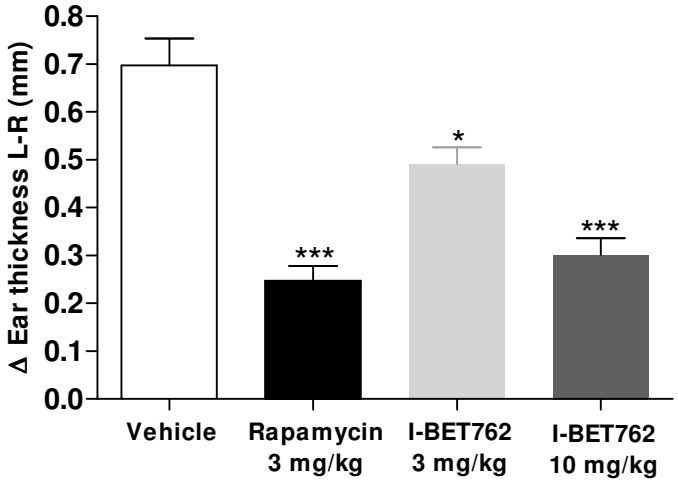


Figure 3. Effect of I-BET762 on ear swelling 24 h post-KLH challenge on Day 8. Results are presented as mean ± SEM (n=10) of the difference between the KLH-injected ear and the NaCl 0.9 %-injected ear. Data were analysed by ANOVA followed by multiple comparisons using Tukey's post-hoc test (* $p < 0.05$, *** $p < 0.001$ versus vehicle).

Conclusions

In this report we have summarized our successful efforts in the optimization of the initial lead GR90698X **7** culminating in the identification of the clinical compound I-BET762, **1**. Introduction and optimization of acetamide substituents in the 3-position of the benzodiazepine core resulted in analogues stable under acid conditions and amenable to enantioselective synthesis. SAR optimization produced I-BET762, **1**, having the best overall potencies in the BRD binding and cellular assays. This compound showed excellent preclinical development properties and progressed into clinical development in oncology during 2012.[6]

Experimental section

General. All commercial chemicals and solvents are reagent grade and were used without further purification unless otherwise specified. All reactions except those in aqueous media were carried out with the use of standard techniques for the exclusion of moisture. Reactions performed under microwave irradiation utilized either a Biotage Initiator or CEM Discover Microwave. Reactions were monitored by thin-layer chromatography on 0.2 mm silica gel plates (ALUGRAM SIL G/UV254, Macherey-Nagel) and visualized with UV light. Final compounds

were typically purified either by flash chromatography on silica gel (E. Merck, 40-63 mm) or by recrystallization. All final compounds analyzed were >95% pure unless otherwise indicated. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker 300 MHz Avance DPX. Chemical shifts are reported in parts per million (ppm, δ units). Splitting patterns are designed as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; bs, broad singlet. High-resolution mass spectra were recorded on a Micromass LCT (TOF) spectrometer coupled to an analytical high performance liquid chromatography (HPLC) was conducted on a XTERRA-MS C18 column (30 × 3 mm id, 2.5 µm) eluting with 0.01 M ammonium acetate in water and 100% acetonitrile (CH_3CN), using the following elution gradient: 0 to 100% CH_3CN over 4 min and 100% CH_3CN over 1 min at 1.1 mL/min at 40 °C. Mass spectra were acquired in either positive or negative ion mode under electrospray ionization (ESI) method. Melting points were measured with a Kofler bench and were uncorrected.

General procedure for the thionation reaction (Method A). Lawesson's reagent (0.6 equiv.) was added to a suspension of amide (1 equiv.) in toluene (0.3 mmol/L). The reaction mixture was heated to reflux for 4 h and then concentrated to dryness. The residue was purified by recrystallization or chromatography on silica gel.

General procedure for the triazole formation 1 (Method B). Hydrazine hydrate (10 equiv.) was added at room temperature to a solution of thioamide (1 equiv.) in THF. After stirring for 3 hours the reaction mixture was concentrated under reduced pressure. The residual oil was dissolved in DCM and washed with water and brine and dried over Na_2SO_4. After filtration the organic phase was concentrated to give the expected hydrazone. The latter was dissolved in a mixture of

EtOH/THF and trimethylorthoacetate (5 equiv.) was added. The reaction mixture was stirred at reflux for 2 h before being concentrated under reduced pressure. The crude product was purified by recrystallization or chromatography on silica gel.

General procedure for the triazole formation 2 (Method C). To a solution of thioamide (1 equiv.) in methanol was added hydrazine monohydrate (1.5 equiv.) at room temperature. The reaction mixture was stirred for 16 hours before being concentrated under reduced pressure. The residue was dissolved in DCM and organics were washed with water, brine and dried over Na_2SO_4. After filtration, the organic phase was concentrated to give the expected hydrazone. The latter was dissolved in dry THF at 0°C, under a nitrogen atmosphere, then DIPEA (1 equiv.) and acetyl chloride (1.05 equiv.) were added and stirred for 1 h. Solvent was removed under reduced pressure and residue was dissolved in acetic acid and stirred at reflux for 30 min. Solvent removed under reduced pressure and the crude product was purified by recrystallization or chromatography on silica gel.

General procedure for the formation of benzodiazepine-2-one (Method D). Step 1: Substituted 2-aminobenzophenone (1 equiv.) and 2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-2-(((benzyloxy)carbonyl)amino)acetic acid[23] **15** (1.5 equiv.) in DCM were cooled to 0°C under nitrogen. EDC (1.5 equiv.) and catalytic 4-DMAP were added at 0°C. The resulting solution was allowed to warm to RT and stirred for 1 h. The reaction was washed with sat. sodium bicarbonate solution and brine. The organic solution was dried over $MgSO_4$ and the solvent removed *in vacuo* to afford the desired product.

Step 2: 7N ammonia in MeOH (100 equiv.) was added to the crude product (1 equiv.) at RT under nitrogen. The resulting solution was stirred at RT for 1 h. The reaction was diluted with EtOAc and washed with 1N NaOH solution. The aqueous was extracted further with EtOAc. The combined organics were dried over $MgSO_4$ and evaporated to give the desired compound

Step 3: Ammonium acetate (5 equiv.) was added to a solution of the previous product (1 equiv.) in glacial acetic acid at RT and the reaction mixture was stirred at RT for 24 h. The solvent was evaporated and the residue co-evaporated with toluene. The mixture was basified with 2N NaOH and extracted with EtOAc and dried over $MgSO_4$ and concentrated *in vacuo*. Purification by trituration from ether afforded the desired benzodiazepine-2-one.

General procedure for amide synthesis (Method E). To a solution of acid (1 equiv.) in THF at RT was added diisopropylethylamine (2 equiv.) followed by HBTU (2 equiv) (HATU could be used as well). The reaction mixture was stirred for 3 h at this temperature and amine (2 equiv.) was added. The mixture was stirred overnight before being concentrated under reduced pressure. The crude material was dissolved in DCM and washed successively with water, 1N NaOH and 1N HCl. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by recrystallization or chromatography on silica gel.

1-Methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-amine 8. To a solution of phenylmethyl (1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-yl)carbamate **7** (4 g, 9.5 mmol) in methanol (100 mL) under nitrogen was added palladium/ carbon catalyst (4 g, 10%) followed by 1,4-cyclohexadiene (6 mL) and the reaction mixture was stirred at RT for 4

hours. The mixture was filtered through Celite and the filtrate was evaporated under reduced pressure to afford the title compound (2.7 g, 98% yield) as a yellow foam which was used directly in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.6 - 7.2 (m, 9 H), 4.9 (br s, 1 H), 2.55 (s, 3 H). LC/MS $(M+H)^+$ = 290, Rt 1.74 min.

***N*-(1-Methyl-6-phenyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)-3-phenylpropanamide 9a**. To a solution of 1-methyl-6-phenyl-4*H*-[1, 2, 4]triazolo[4, 3-*a*][1, 4]benzodiazepin-4-amine **8** (289 mg, 1.0 mmol), and 3-phenylpropanoyl chloride (185 mg, 1.1 mmol, 1.1 equiv.), in DCM (10 mL) was added TEA (1.0 mL, 1.1 mmol). The mixture was stirred at RT overnight and concentrated to dryness. Purification of the residue by flash chromatography on silica gel using DCM/MeOH as eluent (90/10) gave the title compound (10 mg, 2% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.73 (m, 1 H), 7.64 - 7.43 (m, 4 H), 7.43 - 7.16 (m, 6 H), 6.21 (d, J = 8.5 Hz, 1 H), 3.08 (t, J = 7.5 Hz, 2 H), 2.77 (t, J = 7.5 Hz, 2 H), 2.68 (s, 3 H). LC/MS: $(M+H)^+$= 422, Rt 2.42 min.

1-Benzyl-3-(1-methyl-6-phenyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)urea 9b. To a solution of methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-amine **8** (145 mg, 0.5 mmol) in dry DCM (10 mL) are added at room temperature benzylisocyanate (67 mg, 0.5 mmol, 1 equiv.) and DIPEA (129 mg, 1 mmol, 2 equiv.). After stirring overnight, the white precipitate which has formed is filtered off and washed with iPr_2O. The title compound (120 mg, 57% yield) was obtained as a white powder: Mp> 260°C. ^{1}H NMR (300 MHz, DMSO-d6) δ 7.94 - 7.78 (m, 2 H), 7.71 (d, J = 9.2 Hz, 1 H), 7.60 (m, 1 H), 7.56 - 7.39 (m, 6 H), 7.39 -

7.20 (m, 5 H), 7.00 (m, 1 H), 5.82 (d, *J* = 9.2 Hz, 1 H), 4.30 (m, 2 H), 2.59 (s, 3 H). HRMS calculated for $C_{25}H_{23}N_6O$ $(M + H)^+$ 423.1933; found 423.1891. Rt 2.49 min.

***N*-(1-Methyl-6-phenyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)-1-phenylmethanesulfonamide 9c**. To a solution of 1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-amine **8** (100 mg, 0.35 mmol) and pyridine (0.05 mL, 0.7 mmol, 2 equiv.) in dry acetonitrile (1 mL) at 0°C was added benzylsulfonyl chloride (0.07 g, 0.35 mmol, 1 equiv.) in acetonitrile (1 mL). The reaction mixture was stirred at 0°C for 30 min and allowed to warm to RT over 2 h. The solvent was removed *in vacuo* and the residue partitioned between DCM and 2N HCl. The aqueous layer was extracted with DCM and the organic layer was washed with saturated $NaHCO_3$, dried over $MgSO_4$ and concentrated to yield the title compound (108 mg, 70 % yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.74 (m, 1 H), 7.63 - 7.58 (m, 2 H), 7.58 - 7.46 (m, 6 H), 7.44 - 7.38 (m, 2 H), 7.36 - 7.29 (m, 3 H), 6.52 (d, *J* = 8.4 Hz, 1 H), 5.60 (d, *J* = 8.4 Hz, 1 H), 4.73 (d, *J* = 13.6 Hz, 1 H), 4.59 (d, *J* = 13.6 Hz, 1 H), 2.68 (s, 3 H). LCMS: $(M+H)^+$= 444, Rt 2.91 min.

Ethyl (1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-yl)acetate 11.

To a solution of benzodiazepine **10** (1.1 g, 4 mmol) in THF at -78°C was added dropwise a solution of LiHMDS (4 mL, 1M in THF, 4 mmol, 1 equiv.). The reaction mixture was stirred for 15 min at this temperature and a solution of ethyl bromoacetate (444 µL, 4 mmol, 1 equiv.) in THF (5 mL) was added dropwise. The mixture was then allowed to stir from -78°C to RT for 24 h before being quenched with saturated aqueous NH_4Cl. After adding some water, the mixture

was extracted once with a EtOAc/DCM mixture and twice with DCM. The combined organics were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude compound was purified by flash chromatography (silica gel, DCM/MeOH 95/5) and the resulting solid was triturated in hot iPr_2O to give the title compound (690 mg, 48% yield) as a white solid. Mp = 176-177 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.71 (m, 1 H), 7.56 - 7.41 (m, 6 H), 7.41 - 7.33 (m, 2 H), 4.63 (t, *J* = 7.1 Hz, 1 H), 4.24 (q, *J* = 7.2 Hz, 2 H), 3.64 (d, *J* = 7.2 Hz, 2 H), 2.68 (s, 3 H), 1.33 (t, *J* = 7.2 Hz, 3 H). LCMS: $(M+H)^+$= 361, Rt 2.16 min.

(1-Methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-yl)acetic acid 12. To a solution of ester **11** (360 mg, 1 mmol) in a THF/EtOH mixture (20 mL, 1/1) at RT was added 1N NaOH (5 mL, 5 mmol, 5 equiv.) and the reaction mixture was stirred for 1.5 h. 1N HCl (5 mL) was then added dropwise and the resulting white precipitate was filtered and dried under reduced pressure to give the title compound (260 mg, 78% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 12.48 (br s, 1 H), 7.95 - 7.75 (m, 2 H), 7.58 (m, 1 H), 7.53 - 7.45 (m, 3 H), 7.45 - 7.36 (m, 3 H), 4.40 (dd, *J* = 7.7 and 6.8 Hz, 1 H), 3.42 (dd, *J* = 16.8 and 6.8 Hz, 1 H), 3.32 (dd, *J* = 16.8 and 7.7 Hz, 1 H), 2.58 (s, 3 H). LCMS: $(M+H)^+$= 333, Rt 1.37 min.

2-(1-Methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-yl)-*N*-(phenylmethyl)acetamide 13. To a suspension of acid **12** (83 mg, 0.25 mmol) in DMF (2 mL) at RT was added successively HATU (114 mg, 0.3 mmol, 1.2 equiv.), benzylamine (33µL, 0.3 mmol, 1.2 equiv.) and Et_3N (70 µL, 0.5 mmol, 2 equiv.). The reaction mixture was stirred for 16 h and the white precipitate was filtered, washed with water and isopropyl ether. The solid was

then dried under reduced pressure to give the title compound (75 mg, 71% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.63 (m, 1 H), 7.45 - 7.33 (m, 6 H), 7.31 - 7.23 (m, 6 H), 7.20 (m, 1 H), 4.60 (t, J = 7.1 Hz, 1 H), 4.53 (d, J = 14.7 Hz, 1 H), 4.29 (d, J = 14.7 Hz, 1 H), 3.38 (m, 2 H), 2.57 (s, 3 H). LCMS: $(M+H)^+$= 422, Rt 2.22 min. HRMS $(M+H)^+$ calculated for $C_{26}H_{24}N_5O$ 422.1980; found 422.1962.

Benzyl (7-nitro-5-phenyl-2-thioxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate 17a. Method A was used starting from benzyl (7-nitro-5-phenyl-2-thioxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate **16a**.[30] Yield: 8% (brown oil). LCMS: $(M+H)^+$= 446, Rt 3.11 min.

Benzyl (1-methyl-8-nitro-6-phenyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)carbamate 18a. Method B was used. Yield: 65%. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.60 (d, J = 8.1 Hz, 1 H), 8.38 (br s, 1 H), 7.84 (m, 1 H), 7.56 (m, 1 H), 7.49 - 7.32 (m, 8 H), 7.10 (m, 1 H), 5.97 (m, 1 H), 5.22 (s, 2 H), 2.82 (s, 3 H). LCMS: $(M+H)^+$= 469, Rt 3.07 min. HRMS calculated for $C_{25}H_{21}N_6O_4$ $(M+H)^+$: 469.1624, found 469.1586.

Benzyl (7-chloro-5-phenyl-2-thioxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate 17b. Method A was used starting from benzyl (7-chloro-5-phenyl-2-thioxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate **16b**.[31] Yield: 41% (beige solid). LCMS: $(M+H)^+$= 436, Rt 3.72 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.82 (s, 1 H), 8.33 (d, J=8.1 Hz, 1 H), 7.77

(dd, *J* = 9.1, 2.5 Hz, 1 H), 7.58 - 7.44 (m, 6 H), 7.42 - 7.25 (m, 6 H), 7.17 (d, *J* = 7.6 Hz, 1 H), 6.98 (d, *J* = 3.0 Hz, 1 H), 5.25 (d, *J* = 8.1 Hz, 1 H), 5.09 (s, 2 H).

Benzyl (8-chloro-1-methyl-6-phenyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)carbamate 18b. Method C was used. Yield: 31%. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.69 (dd, *J* = 8.8, 2.3 Hz, 1 H), 7.54 (m, 2 H), 7.51 - 7.46 (m, 2 H), 7.46 - 7.30 (m, 7 H), 6.87 (d, *J* = 8.6 Hz, 1 H), 5.93 (d, *J* = 9.1 Hz, 1 H), 5.21 (s, 2 H), 2.65 (s, 3 H). LCMS: $(M+H)^+$= 458, Rt 3.23 min. HRMS $(M+H)^+$ calculated for $C_{25}H_{21}N_5O_2$ 458.1384; found 458.1378.

Benzyl (8-methyl-5-phenyl-2-thioxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate 17c. Method A was used starting from benzyl (8-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate **16c**.[31] Yield: 66% (orange solid). LCMS: $(M+H)^+$= 416, Rt 3.64 min.

Benzyl (1,9-dimethyl-6-phenyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)carbamate 18c. Method C was used. Yield: 38%. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.54 (m, 2 H), 7.49 - 7.23 (m, 13 H), 6.88 (d, *J* = 9.1 Hz, 1 H), 5.92 (d, J = 9.1 Hz, 1 H), 5.20 (s, 2H), 2.67 (s, 3 H), 2.55 (s, 3 H). LCMS: $(M+H)^+$= 438, Rt 3.15 min. HRMS $(M+H)^+$ calculated for $C_{26}H_{24}N_5O_2$ 438.1930; found 438.1934.

Benzyl (7-methoxy-5-phenyl-2-thioxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate 17d. Method A was used starting from benzyl (7-methoxy-2-oxo-5-phenyl-2,3-

dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate **16d**.[31] Yield 89% (brown oil). LCMS: $(M+H)^+$= 432, Rt 2.84 min.

Benzyl (8-methoxy-1-methyl-6-phenyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)carbamate 18d. Method C was used. Yield: 4% (white solid). Mp = 183°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.58 (m, 2 H), 7.52 - 7.30 (m, 9 H), 7.23 (dd, *J* = 8.8 and 2.9 Hz, 1 H), 6.93 (d, *J* = 2.9 Hz, 1 H), 6.89 (d, *J* = 8.8 Hz, 1 H), 5.94 (d, *J* = 9.0 Hz, 1 H), 5.21 (s, 2 H), 3.81 (s, 3 H), 2.66 (s, 3 H). LC/MS: $(M+H)^+$= 454, Rt 2.74 min. HRMS $(M+H)^+$ calculated for $C_{26}H_{24}N_5O_3$ 454.1879; found 454.1809.

Benzyl (5-(2-methoxyphenyl)-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate 16e. Method D was used. Yield: 31%. LC/MS: $(M+H)^+$= 416, Rt 3.13 min.

Benzyl (5-(2-methoxyphenyl)-2-thioxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate 17e. Method A was used starting from benzyl 5-(2-methoxyphenyl)-2-oxo-2,3-dihydro-1*H*-1,4-benzodiazepin-3-ylcarbamate **16e**. Yield: 40% (brown oil). LC/MS: $(M+H)^+$= 432, Rt 3.39 min.

Benzyl (6-(2-methoxyphenyl)-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)carbamate 18e. Method C was used. Yield: 42% yield (off white solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.68 - 7.58 (m, 2 H), 7.47 - 7.28 (m, 9 H), 7.06 (dd, *J* = 7.1 and 7.1 Hz, 1 H), 6.89 (d, *J* = 9.1 Hz, 1 H), 6.79 (d, *J* = 8.1 Hz, 1 H), 5.96 (d, *J* = 9.1 Hz, 1 H), 5.20 (d, *J* = 12.4

Hz, 1 H), 5.18 (d, *J* = 9.1 Hz, 1 H), 3.37 (s, 3 H), 2.66 (s, 3 H). LC/MS: $(M+H)^+$= 454, Rt 3.00 min. HRMS $(M+H)^+$ calculated for $C_{26}H_{24}N_5O_3$ 454.1879; found 454.1785.

Benzyl (5-(3-methoxyphenyl)-2-thioxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate 17f. Method A was used starting from benzyl (5-(3-methoxyphenyl)-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate **16f**.[31] Yield: 50%. LC/MS: $(M+H)^+$= 432, Rt 3.46 min.

Benzyl (6-(3-methoxyphenyl)-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)carbamate 18f. Method C was used. Yield: 24% (off white solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.71 (m, 1 H), 7.55 - 7.45 (m, 3 H), 7.45 - 7.24 (m, 6 H), 7.16 (m, 1 H), 7.01 (m, 2 H), 6.90 (d, *J* = 9.1 Hz, 1 H), 5.94 (d, *J* = 9.1 Hz, 1 H), 5.20 (s, 2 H), 3.83 (s, 3 H), 2.66 (s, 3 H). LC/MS: $(M+H)^+$= 454, Rt 3.05 min.

Benzyl (5-(4-methoxyphenyl)-2-thioxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate 17g. Method A was used starting from benzyl (5-(4-methoxyphenyl)-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate **16g**.[31] Yield: 76% (yellow solid). LC/MS: $(M+H)^+$= 432, Rt 3.53 min. HRMS $(M+H)^+$ calculated for $C_{24}H_{22}N_3O_3S$ 432.1382 found 432.1431.

Benzyl (6-(4-methoxyphenyl)-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)carbamate 18g. Method C was used. Yield: 47% (off white solid). ^{1}H NMR (400 MHz,

$CDCl_3$) δ ppm 7.71 (m, 1 H), 7.57 - 7.44 (m, 5 H), 7.44 - 7.28 (m, 5 H), 6.93 - 6.82 (m, 3 H), 5.90 (d, J = 9.1 Hz, 1 H), 5.20 (s, 2 H), 3.84 (s, 3 H), 2.66 (s, 3 H). LC/MS: $(M+H)^+$= 454, Rt 3.05 min. HRMS $(M+H)^+$ calculated for $C_{26}H_{24}N_5O_3$ 454.1879; found 454.1886.

Benzyl (2-oxo-5-(*p*-tolyl)-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate 16h. Method D was used. Yield: 54% (beige solid). LC/MS: $(M+H)^+$= 400, Rt 3.32 min.

Benzyl (2-thioxo-5-(*p*-tolyl)-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate 17h. Method A was used starting from benzyl (2-oxo-5-(*p*-tolyl)-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate **16h**. Yield: 39%. LC/MS: $(M+H)^+$= 438, Rt 3.16 min.

Benzyl (1-methyl-6-(*p*-tolyl)-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)carbamate 18h. Method C was used. Yield: 49% yield (off white solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.71 (m, 1 H), 7.52 - 7.47 (m, 2 H), 7.47 - 7.29 (m, 8 H), 7.17 (d, J = 8.1 Hz, 2 H), 6.88 (d, J = 8.6 Hz, 1 H), 5.92 (d, J = 8.6 Hz, 1 H), 5.20 (s, 2 H), 2.66 (s, 3 H), 2.38 (s, 3 H). LC/MS: $(M+H)^+$= 438, Rt 3.16 min. HRMS $(M+H)^+$ calculated for $C_{26}H_{24}N_5O_2$ 438.1930; found 438.1852.

4-Fluorobenzyl (4-nitrophenyl) carbonate. A solution of 4-nitrophenylchloroformate (500 mg, 2.5 mmol) in dry DCM (8 mL) was added dropwise to a solution of 4-fluorobenzyl alcohol (0.27 mL, 2.5 mmol) and pyridine (0.21 mL, 2.62 mmol, 1.05 equiv.) at 0°C under nitrogen and the mixture was stirred from 0°C to RT overnight. The mixture was washed with 1N HCl and the

layers separated. The organic extract was dried over $MgSO_4$ and concentrated under reduced pressure. Purification by flash chromatography on silica gel eluting with 17:3 cyclohexane / EtOAc gave the title compound (411 mg, 51% yield) as a colorless gum; LC/MS: $(M+H)^+$= no ion; Rt 3.39 min.

4-Fluorobenzyl (1-methyl-6-phenyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)carbamate 19a. A solution of 1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-amine **8** (70 mg, 0.24 mmol), and 4-fluorobenzyl (4-nitrophenyl) carbonate (70 mg, 0.24 mmol) and TEA (34 µL, 0.24 mmol) in acetonitrile (4 mL) was stirred at reflux for 36 h before being concentrated to dryness. The residue was dissolved in EtOAc and the organic phase was washed respectively with water, sat. $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel using EtOAc/cyclohexane as eluent (8/1) gave the title compound **19a** (48 mg, 45% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.73 (m, 1 H), 7.58 - 7.44 (m, 6 H), 7.43 - 7.34 (m, 4 H), 7.06 (m, 2 H), 6.90 (d, J = 9.0 Hz, 1 H), 5.93 (d, J = 9.0 Hz, 1 H), 5.16 (s, 2 H), 2.67 (s, 3 H). LC/MS: $(M+H)^+$= 442, Rt 3.07 min.

3-Fluorophenyl (1-methyl-6-phenyl-4H-benzo[f][1,2,4]triazolo[4,3-a][1,4]diazepin-4-yl)carbamate 19b. To a solution of amine **8** (20 mg, 0.069 mmol) in THF (1 mL) at 0°C under nitrogen was added a solution of triphosgene (12 mg, 0.036 mmol) and triethylamine (20 µL, 0.14 mmol) in THF (1 mL) and the mixture was allowed to warm to room temperature and stirred for 10 min. A solution of 3-fluorophenol (12 mg, 0.107 mmol) in THF was then added at

0°C and the mixture was allowed to warm to RT for 1 h. The solvent was then evaporated under reduced pressure and the residue was purified by MDAP affording the title compound **19b** (10.4 mg, 35% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.76 (m, 1 H), 7.92-7.88 (m, 1 H), 7.87 - 7.81 (m, 1 H), 7.63-7.5 (m, 4 H), 7.48-7.41 (m, 4 H), 7.13-7.01 (m, 3 H), 5.74 (d, J = 8.6 Hz, 1 H), 2.50 (s, 3 H). LC/MS: $(M+H)^+$= 428, Rt 6.84 min.

Ethyl (1-methyl-6-phenyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)carbamate 19c. To a solution of 1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepin-4-amine (140 mg, 0.48 mmol) in dry DCM (10 mL) and pyridine (500 µL) was added ethyl chloroformate (46 µL, 0.48 mmol) and the solution was stirred overnight at room temperature. A further equivalent of ethylchloroformate was added and the reaction mixture was allowed to stir at room temperature for an additional 3 hours. The reaction mixture is concentrated *in vacuo*, dissolved in DCM (150 mL) and washed twice with 1N HCl (30 mL). The organic phase was washed with sat. $NaHCO_3$ (30 mL) and brine (30 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Addition of diethyl ether gave a precipitate which was filtered to give the title compound **19c** (108 mg, 62% yield) as a pale yellow solid. Mp = 198-202°C. LCMS: $(M+H)^+$= 362, Rt 2.13 min. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (m, 1 H), 7.50-7.34 (m, 6 H), 7.29 (m, 2 H), 6.67(d, J = 9.1 Hz, 1 H), 5.84 (d, J = 9.1 Hz, 1 H), 4.13 (q, J = 7.1 Hz, 2 H), 2.58 (s, 3 H), 1.23 (t, J = 7.1 Hz, 3 H). HRMS $(M+H)^+$ calculated for $C_{20}H_{20}N_5O_2$ 362.1539 found 362.1548.

(+)-Ethyl 1-methyl-6-phenyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-ylcarbamate (+)-19c and (-)-ethyl 1-methyl-6-phenyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-

ylcarbamate (-)-19c. Enantiomers of ethyl (1-methyl-6-phenyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)carbamate **19c** (260 mg) were separated using a 1" x 25 cm Chiralpak AD column, elution with EtOH/Heptane (80:20), flow rate 15 mL/min. The title enantiomer **(+)-19c** eluted at Rt 9.5 min (125 mg) as the first eluted isomer $[a]_D^{20}$ = + 46.9, c = 0.738 (g/100 mL) in MeOH. The second enantiomer **(-)-19c** eluted at Rt 13.5 min.

Benzyl (5-(4-chlorophenyl)-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate 20. Method D was used. Yield: 63% (white solid). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 9.48 (s, 1 H), 7.60 - 7.45 (m, 3 H), 7.45 - 7.25 (m, 8 H), 7.25 - 7.14 (m, 2 H), 6.67 (d, *J* = 8.1 Hz, 1 H), 5.33 (d, *J* = 8.1 Hz, 1 H), 5.18 (s, 2 H). LC/MS: $(M+H)^+$= 420, Rt 2.83 min.

3-Amino-5-(4-chlorophenyl)-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one 21. To a suspension of benzyl (5-(4-chlorophenyl)-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate **20** (940 mg, 2.2 mmol) in AcOH (5 mL) was added HBr 37% in AcOH (5 mL). The reaction mixture was stirred for 30 min at 80°C before being cooled down and diluted with diisopropyl ether. The resulting solid was filtered and stirred in a saturated solution of $NaHCO_3$ for 30 min. The solid was filtered, washed with water and dried under reduced pressure to give the title compound (590 mg, 92% yield) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-*d*6) δ ppm 10.46 (br s, 1 H), 7.36 (m, 1 H), 7.31 - 7.22 (m, 4 H), 7.11 - 6.91 (m, 3 H), 3.99 (s, 1 H), LC/MS: $(M-H)^-$= 284, Rt 1.80 min.

Ethyl (5-(4-chlorophenyl)-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate 22. To a suspension of 3-amino-5-(4-chlorophenyl)-1*H*-benzo[*e*][1,4]diazepin-2(3*H*)-one **21** (590 mg, 2.0 mmol) and triethylamine (317 µL, 2.2 mmol, 1.1 equiv.) in THF (10 mL) at 0°C was added ethyl chloroformate (217 µL, 2.2 mmol, 1.1 equiv.). The reaction mixture was stirred for 2 h from 0°C to RT before being concentrated to dryness. The residue was dissolved in DCM washed with water, dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel eluting with DCM/MeOH : 99/1 gave the title compound (610 mg, 83% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-*d*6) δ ppm 10.88 (s, 1 H), 8.21 (d, *J* = 8.7 Hz, 1 H), 7.64 (m, 1 H), 7.58 - 7.43 (m, 4 H), 7.38 - 7.20 (m, 3 H), 5.01 (d, *J* = 8.5 Hz, 1 H), 4.02 (q, *J* = 7.0 Hz, 2 H), 1.19 (t, *J* = 7.0 Hz, 3 H). LC/MS: $(M+H)^+$= 358, Rt 2.31 min.

Ethyl (5-(4-chlorophenyl)-2-thioxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate 23. Method A was used starting from ethyl (5-(4-chlorophenyl)-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)carbamate **22**. Yield: 96% (yellow solid). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 11.06 (s, 1 H), 7.47 - 7.27 (m, 3 H), 7.27 - 7.01 (m, 5 H), 6.95 (d, *J* = 8.4 Hz, 1 H), 5.38 (d, *J* = 8.3 Hz, 1 H), 4.09 (m, 2 H), 1.20 (t, *J* = 7.1 Hz, 3 H). LC/MS: $(M+H)^+$= 374, Rt 2.59 min.

Ethyl (6-(4-chlorophenyl)-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)carbamate 19d. Method C was used. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.73 (m, 1 H), 7.56

- 7.44 (m, 5 H), 7.37 - 7.32 (m, 2 H), 6.77 (d, *J* = 9.0 Hz, 1 H), 5.91 (d, *J* = 9.0 Hz, 1 H), 4.21 (q, *J* = 7.1 Hz, 2 H), 2.67 (s, 3 H), 1.31 (t, *J* = 7.1 Hz, 3 H). LC/MS: $(M+H)^+$= 396, Rt 2.16 min.

(*S*)-Methyl 2-(5-(4-chlorophenyl)-7-methoxy-2-oxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)acetate 24. To a solution of the crude (*S*)-methyl 3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-((2-(4-chlorobenzoyl)-4-methoxyphenyl)amino)-4-oxobutanoate in DCM (500 mL) was added Et_3N (500 mL, 3.65 mol, 18 equiv.) and the resulting mixture was refluxed for 24 h before being concentrated. The resulting crude amine was dissolved in 1,2-DCE (1.5 L) and AcOH (104 mL, 1.8 mol, 9 equiv) was added carefully. The reaction mixture was then stirred at 60°C for 2 h before being concentrated *in vacuo* and dissolved in DCM. The organic layer was washed with 1N HCl and the aqueous layer was extracted with DCM (x3). The combined organic layers were washed twice with water, and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude solid was recrystallised in CH_3CN to give the title compound (51 g) as a pale yellow solid. The filtrate could be concentrated and recrystallised in CH_3CN to give to another 10 g of the title compound (total: 61 g, 69% yield based on recovered aminobenzophenone). R_f = 0.34 (DCM/MeOH:95/5). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.73 (br s, 1 H), 7.51 (m, 2 H), 7.36 (m, 2 H), 7.14-7.08 (m, 2 H), 6.76 (m, 1 H), 4.18 (dd, *J* = 7.0 and 7.0 Hz, 1 H), 3.75 (s, 3 H), 3.74 (s, 3 H), 3.42 (dd, *J* = 16.9 and 7.0 Hz, 1 H), 3.20 (dd, *J* = 16.9 and 7.0 Hz, 1 H). LC/MS: $(M+H)^+$= 373, Rt 2.76 min..

(*S*)-Methyl 2-(5-(4-chlorophenyl)-7-methoxy-2-thioxo-2,3-dihydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)acetate 25. A suspension of P_4S_{10} (53 g, 120 mmol, 1.8 equiv.) and

Na_2CO_3 (12.6 g, 120 mmol, 1.8 equiv.) in 1,2-DCE (1 L) at RT was stirred for 2 h before amide **24** (24.7 g, 66.3 mmol) was added. The resulting mixture was stirred at 65°C for 4 h and at room temperature overnight. The residue was treated with a sat. $NaHCO_3$ solution and extracted with DCM. The organic phase was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The oily residue was crystallized from DCM/diisopropyl ether to give the title compound **25** (17.3 g, 67% yield) as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.53 - 7.44 (m, 2 H), 7.38 - 7.31 (m, 2 H), 7.18 (d, *J* = 8.9 Hz, 1 H), 7.11 (dd, *J* = 8.9 and 2.8 Hz, 1 H), 6.77 (d, *J* = 2.8 Hz, 1 H), 4.39 (dd, J = 7.0 and 6.8 Hz, 1 H), 3.76 (s, 3 H), 3.75 (s, 3 H), 3.66 (dd, *J* = 17.0 and 6.8 Hz, 1 H), 3.38 (dd, *J* = 17.0 and 7.0 Hz, 1 H). LC/MS: $(M+H)^+$= 389, Rt 3.31 min.

Methyl 2-((4*S*)-6-(4-chlorophenyl)-8-methoxy-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)acetate 26. Method C was used with temperature kept between 5 and 15°C when reacting with hydrazine.[25] Yield: 86% (yellow powder). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54-7.47 (m, 2 H), 7.40 (d, *J* = 8.8 Hz, 1 H), 7.37-7.31 (m, 2 H), 7.22 (dd, *J* = 2.8 and 8.8 Hz, 1 H), 6.89 (d, *J* = 2.8 Hz, 1 H), 4.61 (dd, *J* = 6.4 and 7.8 Hz, 1 H), 3.82 (s, 3 H), 3.78 (s, 3 H), 3.66 (dd, *J* = 7.8 and 16.9 Hz, 1 H), 3.60 (dd, *J* = 6.4 and 16.9 Hz, 1 H), 2.62 (s, 3 H). LC/MS: $(M+H)^+$= 411, Rt 2.88 min. HRMS $(M+H)^+$ calculated for $C_{21}H_{20}{}^{35}ClN_4O_3$ 411.1229; found 411.1245.

2-((4S)-6-(4-Chlorophenyl)-8-methoxy-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)acetic acid 27. To a solution of methyl 2-((4*S*)-6-(4-chlorophenyl)-8-

methoxy-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)acetate **26** (28 g, 68 mmol) in THF (450 mL) at RT was added 1N NaOH (136 mL, 136 mmol, 2 equiv.). The reaction mixture was stirred at this temperature for 5 h before being quenched with 1N HCl (136 mL) and concentrated *in vacuo*. THF was removed under reduced pressure and the remaining aqueous layer was extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give, after recrystallisation in acetonitrile, the title compound (23.9 g, 89% yield) as a pale yellow powder. $[a]_D^{20}$ = +59.0 (*c* 1.099 in MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 - 7.48 (m, 2 H), 7.41 (d, *J* = 8.9 Hz, 1 H), 7.38 - 7.31 (m, 2 H), 7.22 (dd, *J* = 8.9 and 2.9 Hz, 1 H), 6.90 (d, *J* = 2.9 Hz, 1 H), 4.59 (dd, *J* = 6.9 and 6.9 Hz, 1 H), 3.81 (s, 3 H), 3.70 (dd, *J* = 25.7 and 6.9 Hz, 1 H), 3.61 (dd, *J* = 25.7 and 6.9 Hz, 1 H), 2.63 (s, 3 H). LC/MS: $(M+H)^+$= 397, Rt 2.11 min. Chiral HPLC: column: chiralpak AD RH 150x4.6 mm 10µm; mobile phase: 65/35, H_2O (10% NH_4OAc)/MeOH; Flow rate : 0.5 mL/min; T = 40 °C; UV wavelength: 210 and 254 nm. The title compound eluted at 10.33 min.

2-((4*S*)-6-(4-Chlorophenyl)-8-methoxy-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)-*N*-(4-fluorophenyl)acetamide 28a. Method E was used. Yield: 47% (pale yellow solid). Mp = 252°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 9.21 (s, 1 H), 7.58 - 7.46 (m, 4 H), 7.41 (d, *J* = 8.9 Hz, 1 H), 7.33 (d, *J* = 8.5 Hz, 2 H), 7.22 (dd, *J* = 9.0 and 2.8 Hz, 1 H), 6.95 (m, 2 H), 6.88 (d, *J* = 2.8 Hz, 1 H), 4.67 (dd, *J* = 8.5, 5.8 Hz, 1 H), 3.82 (m, 1 H), 3.81 (s, 3 H), 3.53 (dd, *J* =14.3 and 5.7 Hz, 1 H), 2.64 (s, 3 H). HRMS $(M+H)^+$ calculated for $C_{26}H_{22}{}^{35}ClFN_5O_2$ 490.1446; found 490.1399. LC/MS: $(M+H)^+$= 490, Rt 3.17 min.

2-((4*S*)-6-(4-Chlorophenyl)-8-methoxy-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)-*N*-(thiazol-2-yl)acetamide 28b. Method E was used. Yield: 43% (white solid). Mp = 182°C (becomes gummy). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 12.42 (s, 1 H), 7.81 (d, *J* = 9.1 Hz, 1 H), 7.56 - 7.44 (m, 5 H), 7.40 (dd, *J* = 9.1 and 2.9 Hz, 1 H), 7.20 (d, *J* = 3.6 Hz, 1 H), 6.90 (d, *J* = 2.9 Hz, 1 H), 4.62 (t, *J* = 7.2 Hz, 1 H), 3.80 (s, 3 H), 3.61 (d, *J* = 7.2 Hz, 2 H), 2.54 (s, 3 H). HRMS $(M+H)^+$ calculated for $C_{23}H_{20}{}^{35}ClN_6O_2S$ 479.1057; found 479.1088. LC/MS: $(M+H)^+$= 479, Rt 2.93 min.

2-((4*S*)-6-(4-Chlorophenyl)-8-methoxy-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)-*N*-(pyridin-2-yl)acetamide 28c. Method E was used. Yield: 34% (pale yellow powder). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 9.19 (br s, 1 H), 8.35 (m, 1 H), 8.18 (d, *J* = 8.4 Hz, 1 H), 7.70 (m, 1 H), 7.56 (d, *J* = 8.5 Hz, 2 H), 7.41 (d, *J* = 8.9 Hz, 1 H), 7.34 (d, *J* = 8.5 Hz, 2 H), 7.22 (dd, *J* = 8.9 and 2.9 Hz, 1 H), 7.05 (dd, *J* = 6.7 and 5.2 Hz, 1 H), 6.88 (d, *J* = 2.7 Hz, 1 H), 4.68 (dd, *J* = 7.6 and 6.3 Hz, 1 H), 3.81 (s, 3 H), 3.76 - 3.58 (m, 2 H), 2.64 (s, 3 H). HRMS $(M+H)^+$ calculated for $C_{25}H_{22}{}^{35}ClN_5O_2$ 473.1493; found 473.1508.

2-((4*S*)-6-(4-Chlorophenyl)-8-methoxy-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)-*N*-(pyridin-3-yl)acetamide 28d. Method E was used. Yield: 54% (pale yellow powder).^{1}H NMR (300 MHz, DMSO-*d*6) δ ppm 10.55 (s, 1 H), 8.79 (s, 1 H), 8.27 (d, *J* = 4.0 Hz, 1 H), 8.06 (d, *J* = 8.0 Hz, 1 H), 7.81 (d, *J* = 8.9 Hz, 1 H), 7.52 (d, *J* = 8.5 Hz, 2 H), 7.47 (d, *J* = 8.5 Hz, 2 H), 7.43 - 7.28 (m, 2 H), 6.89 (d, *J* = 2.5 Hz, 1 H), 4.58 (t, *J* = 7.0 Hz, 1 H), 3.62

- 3.42 (m, 2 H), 2.55 (s, 3 H). HRMS $(M+H)^+$ calculated for $C_{25}H_{22}{}^{35}ClN_5O_2$ 473.1493; found 473.1540. LC/MS: $(M+H)^+$= 473, Rt 2.75 min.

2-((4*S*)-6-(4-Chlorophenyl)-8-methoxy-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)-*N*-(cyclopropylmethyl)acetamide 28e. Method E was used. Yield: 51%. Mp = 128°C (becomes gummy). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.55 - 7.47 (m, 2 H), 7.42 - 7.30 (m, 3 H), 7.20 (dd, *J* = 8.9 and 2.8 Hz, 1 H), 6.86 (d, *J* = 2.8 Hz, 1 H), 6.51 (m, 1 H), 4.62 (t, *J* = 7.0 Hz, 1 H), 3.80 (s, 3 H), 3.51 (dd, *J* = 14.3 and 7.0 Hz, 1 H), 3.36 (dd, *J* = 14.3 and 7.0 Hz, 1 H), 3.25 (m, 1 H), 3.08 (m, 1 H), 2.62 (s, 3 H), 1.09 - 0.90 (m, 1 H), 0.58 - 0.47 (m, 2 H), 0.28 - 0.17 (m, 2 H). HRMS $(M+H)^+$ calculated for $C_{24}H_{25}{}^{35}ClN_5O_2$ 450.1697; found 450.1613.

2-((4*S*)-6-(4-Chlorophenyl)-8-methoxy-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)-*N*-ethylacetamide 1. Method E was used. Yield: 47% (white solid). R_f = 0.48 (DCM/MeOH:90/10). Mp > 140°C (becomes gummy). α_D = +88.1 (*c* 1.0015/ MeOH). ^{1}H RMN (300 MHz, $CDCl_3$) δ 7.53 - 7.47 (m, 2 H), 7.39 (d, *J* = 8.9 Hz, 1 H), 7.37- 7.31 (m, 2 H), 7.20 (dd, *J* = 8.9 and 2.9 Hz, 1 H), 6.86 (d, *J* = 2.9 Hz, 1 H), 6.40 (m, 1 H), 4.62 (m, 1 H), 3.80 (s, 3 H), 3.51 (dd, *J* = 14.1 and 7.3 Hz, 1 H), 3.46 - 3.21 (m, 3 H), 2.62 (s, 3 H), 1.19 (t, *J* = 7.3 Hz, 3 H). HRMS $(M+H)^+$ calculated for $C_{22}H_{23}{}^{35}ClN_5O_2$ 424.1540; found 424.1525. Chiral HPLC: column: chiralpak AD 250x4.6 mm 10µm; mobile phase: 60/40, EtOH / Hexane; Flow rate: 1.0 mL/min; UV wavelength: 210 and 254 nm. The title compound eluted at 5.76 min.

2-((4*S*)-6-(4-Chlorophenyl)-9-methoxy-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)-*N*-ethylacetamide 29. Method E was used. Yield: 47% (off white powder). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.45 (m, 2 H), 7.37 - 7.31 (m, 3 H), 6.97 (dd, *J* = 8.8 and 2.5 Hz, 1 H), 6.92 (d, *J* = 2.5 Hz, 1 H), 6.37 (m, 1 H), 4.62 (dd, *J* = 7.2 and 7.0 Hz, 1 H), 3.94 (s, 3 H), 3.52 (dd, *J* = 13.9 and 7.2 Hz, 1 H), 3.44 - 3.21 (m, 3 H), 2.66 (s, 3 H), 1.19 (t, *J* = 7.3 Hz, 3 H). HRMS $(M+H)^+$ calculated for $C_{22}H_{23}{}^{35}ClN_5O_2$ 424.1540; found 424.1527.

***N*-Ethyl-2-((4*S*)-6-(3-fluorophenyl)-8-methoxy-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)acetamide 30**. Method E was used. Yield: 61%. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.55 (m, 1 H), 7.40 - 7.23 (m, 4 H), 7.17 (m, 1 H), 6.93 (d, *J* = 2.6 Hz, 1 H), 4.74 (m, 1 H), 3.83 (s, 3 H), 3.52 (m, 1 H), 3.45 - 3.23 (m, 3 H), 2.81 (br s, 3 H), 1.20 (t, *J* = 7.3 Hz, 3 H). LC/MS: $(M+H)^+$= 408, Rt 2.50 min.

Butyl 2-((4*S*)-6-(4-chlorophenyl)-8-methoxy-1-methyl-4*H*-benzo[*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)acetate 28g. Method E was used. Yield: 85% (yellow foam). ^{1}H RMN (300 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.4 Hz, 2 H), 7.40 (d, *J* = 8.9 Hz, 1 H), 7.35 (d, *J*= 8.4 Hz, 2 H), 7.22 (dd, *J* = 8.9 and 2.9 Hz, 1 H), 6.89 (d, *J* = 2.9 Hz, 1 H), 4.61 (dd, *J* = 8.5 and 5.6 Hz, 1 H), 4.19 (t, *J* = 6.5 Hz, 2 H), 3.85 (s, 3 H), 3.67 (dd, *J* = 16.8 and 8.5 Hz, 1 H), 3.57 (dd, *J* = 16.8 and 5.7 Hz, 1 H), 2.63 (s, 3 H), 1.66 (m, 2 H), 1.42 (m, 2 H), 0.95 (t, *J* = 7.3 Hz, 3 H). LC/MS: $(M+H)^+$= 453, Rt = 3.34 min. HRMS $(M+H)^+$ calculated for $C_{24}H_{26}{}^{35}ClN_4O_3$ 453.1693; found 453.1696.

ASSOCIATED CONTENT

Supporting Information. Additional text describing biological methods and chemistry experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

Crystallography. Crystal structures of BRD4 bound to **7** and **1** are available as entries 2yel and 3p5o respectively.[16a,5] Structures of BRD4 bound to Etizolam and **31** have been deposited with the PDB (accession codes to be added when manuscript is accepted).

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

Notes

All studies involving the use of animals were conducted after review by the GlaxoSmithKline (GSK) Institutional Animal Care and Use Committee and in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals.

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ABBREVIATIONS

BET: bromodomain and extra-terminal; NUT: nuclear protein in testis; BRD2/3/4, bromodomain-containing protein 2/3/4; BRDT: bromodomain, testis-specific; MLL: mixed lineage leukemia; FP: Fluorescence Polarization; IL-6, interleukin-6; PBMC: Peripheral Blood Mononuclear Cell; NT: Not Tested; EDCI: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; DMAP: 4-Dimethylaminopyridine; Bt: Benzotriazole; DIPEA: Di-isopropylethylamine; 1,2-DCE: 1,2-Dichloroethane

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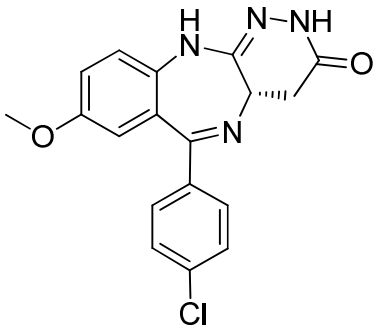

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