Discovery and Development of Bromodomain Inhibitors

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Abstract: Bromodomains is one of the epigenetic readers that can recognize and bind to acetylated lysine residues. Since the discovery of the first selective bromodomain inhibitor, extensive research was conducted in order to design novel potent bromodomain inhibitors. In this review, we are summarizing some of the work that has done is in this area.

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1. Introduction

Targeting protein-protein interactions (PPI) has always been considered a big challenge for medicinal chemists. The discovery of inhibitors for the bromodomains is believed to be an excellent opportunity for targeting PPI. Bromodomain (BRD) is an epigenetic reader that can recognize and bind to the acetylated lysine (Kac). BRDs are a family of evolutionarily conserved modules of approximately 110 residues that first identified in Brahma gene of Drosophila. BRD consists of four α-helices (α_A, α_B, α_C and α_Z) that are packed to each other in an antiparallel mode. The α-helices are joined by two loops, the first connects α_Z with α_A called ZA loop while the other BC loop connects α_B to α_C (**Figure 1**). Such structure of the four helices forms a deep hydrophobic pocket, extended by the ZA and BC loops, thus forming the binding site for the Kac (**Figure 2**). This hydrophobic pocket has a conserved water network that has an important role in binding with Kac[1,2]. Most BRDs utilize their conserved asparagine residue (Asn140 in bromodomain containing protein4-first bromodomain [BRD4-BD1]) located in BC loop, to form a hydrogen bond with carbonyl group of Kac. Another important water-mediated hydrogen bond between carbonyl of Kac and a conserved tyrosine residue (Tyr-97 in BRD4-BD1). Also the methyl group of Kac fits well in a small hydrophobic pocket. Despite their structural similarity, selectivity of BRDs to Kac of different proteins was detected and studied in order to develop selective probes and inhibitors. First, the electrostatic environment around the Kac binding site diverges greatly from strong positively charged field in some to strong negative one in others so making the different bromodomains more selective to Kac according to the neighboring residues.[1,3] In addition, ZA channel (channel between ZA loop and α_A) can be employed to increase the potency and selectivity. In addition, WPF shelf could serve as a way for selective inhibition of different bromodomains in spite of being non conserved in some bromodomains. Additionally, a residue above the WPF shelf - called the gatekeeper- controls the access to the WPF residues. The variability of gatekeeper residues among the various bromodomains can offer a rationale for the design of selective bromodomain inhibitors, according to the steric properties of the gate keeper amino acid. [4,5,6]

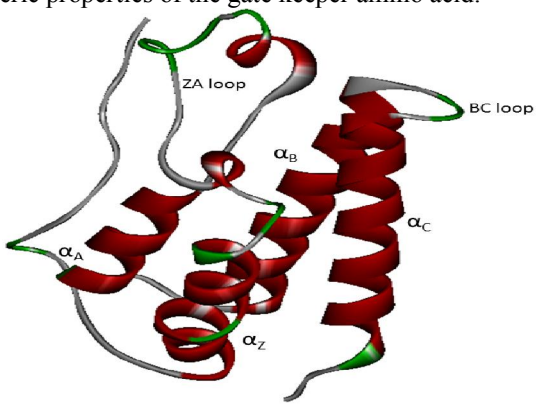


Figure 1: 3D structure of bromodomain

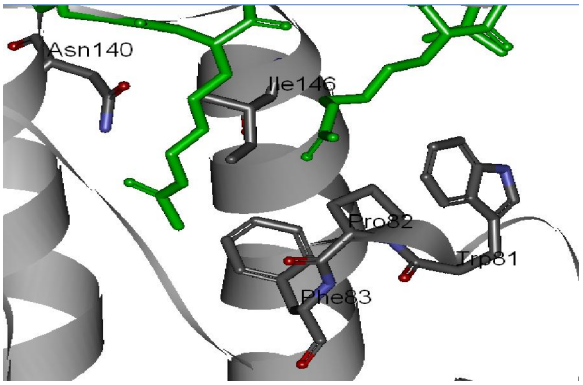


Figure 2: this figure shows 2 Kac (appear in green) while binding to BD1 of BRD4(PDB ID = 3UVW)

There are 61 different BRDs in human found in 46 proteins that are grouped in 8 families according to their structural similarity (**Figure 3**).[7] Recently, novel inhibitors of bromodomains have been developed through several strategies, such as high throughput screening (HTS) techniques, fragment-based drug discovery (FBDD), computational techniques, rational design or combinations of these. Number of inhibitors discovered for Family II [bromodomain and extra-terminal domain (BET) family], are more than the total number of inhibitors discovered for all other families. Thus we can classify the inhibitors developed to BET inhibitors and non-BET inhibitors.

family	members of the family
I	PCAF, GECR2, FALZ, GCN5L2
II	BRD2, BRD3, BRD4, BRDT
III	CREBBP, BAZ1B, BRD8B, BRWD3,EP300, PHIP
IV	BRD1, BRD7, BRD9, ATAD2, BRPF1,BRPF3 KIAA1240
V	BAZ2A, BAZ2B, LOC93349, TRIM33, TRIMM66
VI	MLL, TRIM28
VII	PRKCBP1, TAF1, TAF1L
VIII	PB1, SMARCA2, SMARCA4

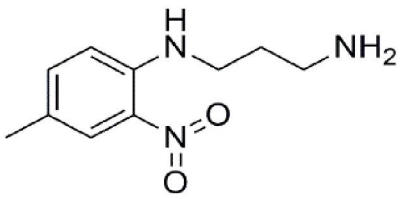
Figure 3: families of bromodomain containing proteins and the most important members of them

In this review, we will discuss some of the new inhibitors discovered for some bromodomains.

2. Non-BET proteins and their inhibitors:

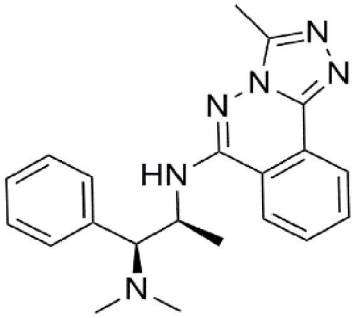
2.1. P300/CBP-associated factor (PCAF) inhibitors [Family I]:

PCAF is a human transcriptional coactivator. It has a bromodomain which can recognize Kac on specific sites on histone tails.[8] PCAF has been connected to some human disorders while its role in HIV infections is the most prominent. PCAF bromodomain is capable of recognizing Kac of human immunodeficiency virus (HIV) Tat. The Tat /PCAF association is crucial for HIV replication. Thus, PCAF bromodomain offers an alternative way for treatment of HIV by targeting the host's protein rather than the rapidly mutating HIV proteins. One of the first trials to find selective BRD inhibitors, was compound **1** (**Figure 4**).[9] Moreover, compound **2 (Figure 4)** was designed to be a selective probe for PCAF-BRD.[10]



1

IC_{50} (PCAF) = 1.6 μM



2

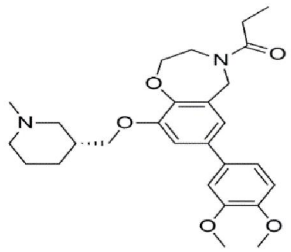
IC_{50} (PCAF) = 126 nM

Figure 4: PCAF inhibitors

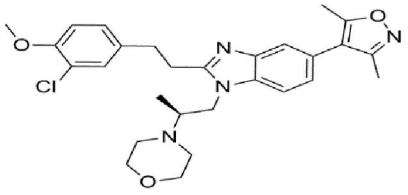
2.2. cAMP response element binding protein (CBP) BRD inhibitors [Family III]:

CBP (CREBBP or KAT3A) and P300 (EP300 or KAT3B) are multi-domain proteins and closely related HATs. They coordinate the construction of a multicomponent transcription factor complex. Their BRD is required for substrate specificity and transcriptional activity.[11] Their BRD can recognize and bind to Kac of p53 after DNA damage so it is important for DNA damage control.[12] Also many studies were performed to identify the role of CBP/P300 in cancer.[13,14,15,16,17,18] Therefore the multi-domain CBP/P300 is a target to develop new anti-cancer agents. One approach to inhibit CBP/P300 is by developing inhibitors for their BRDs. Examples for selective CBP-BRD inhibitors are compounds **3**[19], **4**[20], **5**[21] and **6**[22] **(Figure 5).** All compounds share Kac

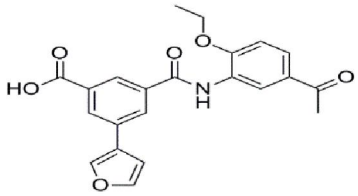
mimetic structure that is able to make the essential 2 hydrogen bonds with the binding site of the receptor.



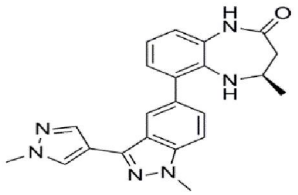
3
IC_{50} (CBP) = 170 nM



4
IC_{50} (CBP) = 21 nM



5
IC_{50} (CBP) = 170 nM

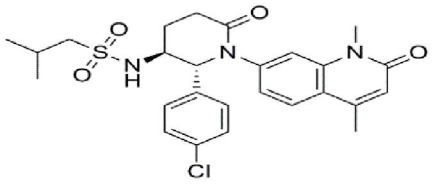


6
IC_{50} (CBP) = 30 nM

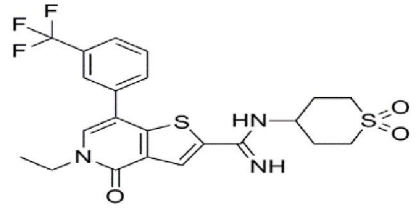
Figure 5: CBP inhibitors

2.3. Bromodomain containing protein 7/9 (BRD7 and BRD9) [Family IV]:

Both BRD7 and BRD9 associate the SWI/SNF complexes PBAF and BAF, respectively. The SWI/SNF complexes are incorporated in the gene transcription and DNA repair.[23] About 20% of human cancers have SWI/SNF mutations. But the role of BRD7 and BRD9 was barely known due to the lack of selective inhibitors.[24] Thus research was performed for the discovery of selective BRD7/9 inhibitors. First selective BRD7/9 inhibitor over other BRDs, was compound **7** (**Figure 6**).[25] Compound **8** (**Figure 6**) exhibitsan IC_{50} = 50 nM with 100 folds more selective for BRD9 over BRD4.[26] Also fragment **9** (**Figure 6**) provides a novel template for the design of new potent and selective BRD7/9 inhibitors.[27]



7
ITC k_D = 99 nM



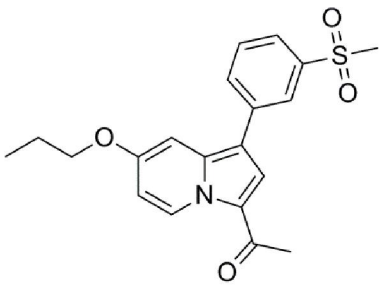
8
IC_{50} (BRD9) = 50 nM

9
IC_{50} (BRD9) = 278 nM

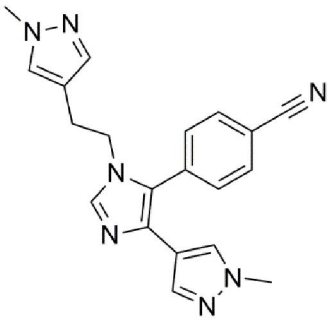
Figure 6: BRD7/9 inhibitors

2.4. Bromodomain adjacent to zinc finger domain 2A/B (BAZ2A/B) [Family V]:

The function of BAZ2A/B is not well studied. However, it is a part of nuclear remodeling complex (NoRC).[28] Also, elevated levels of BAZ2A was observed in prostate cancer.[29] This result has pushed the research forward to develop selective BAZ2A/B inhibitors [for example; compounds **10**[30] and **11**[31] (**Figure 7**)].



10
BAZ2A K_d = 260 nM
BAZ2B K_d = 140 nM



11
BAZ2A K_d = 130 nM
BAZ2B K_d = 180 nM

Figure 7: BAZ2A/B inhibitors

2.5. SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily A (SMARCA4):

SMARCA4 is also known as Brahma-related gene 1 (BRG1). It is found to be connected to different tumors especially lung cancer.[32,33] Compound **12 (Figure 8)** has been discovered by Pfizer however, the design and the optimization steps have not been published yet.[32]

12
IC_{50} (SMARCA4) = 89 nM

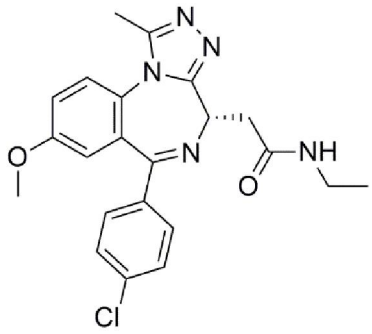
Figure 8: SMARCA4 inhibitor

3. BET family and their inhibitors:

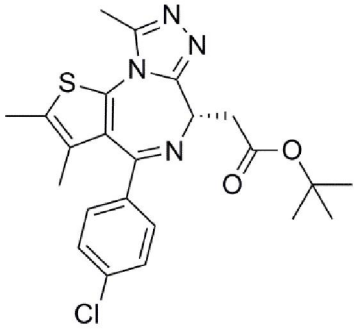
BET Family is characterized by having two bromodomains (BDs) and an extra-terminal (ET) domain. The family has four proteins; BRD2, BRD3, BRD4 and bromodomain testis-associated protein [BRDT].[34,35] They are vital players in the transcription regulation. It is characterized by remaining associated with the chromosomes during mitosis while other transcription factors are released in the cytoplasm. Therefore, it could have a function in transferring the transcriptional memory during cell division.[36,37,38] BET inhibition is found to be a therapeutic approach for many human disorders like COPD[39], atherosclerosis[40,41,42], inflammatory disorders.[40,43,44], some viral infections[4546,47] and cancer. Yet, its anticancer activity is found to be the most prominent. Its mechanism of action can be summarized as the following; first G1 cell cycle arrest, second inhibition of oncogene transcription leading to apoptosis and finally, inhibition of the pathways that preserve the tumor supportive environment.[48]Recently, it is found that BET inhibitors could be used as an adjunct therapy with levodopa.[49]

In 2010, The first two potent BET inhibitors discovered are structurally related to diazepine (compound **13** [I-Bet762] and compound **14** [JQ1], **Figure 9**).[44,50] Since after, the academic and industrial interest for the development of selective BET inhibitors started. Shortly after that, two new potent BET inhibitors were discovered to expand the chemical space that could be used for the design of the new inhibitors (**15** & **16 Figure 9**).[51,52] All designed inhibitors share similar binding mode to Kac binding with the bromodomains. Most of the inhibitors conserve a hydrogen bond with the conserved Asn

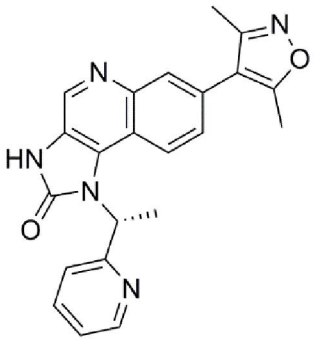
(Asn 140 in BRD4-BD1) and a water-mediated hydrogen bond with Tyr (Tyr97 in BRD4-BD1). Additionally, they share a hydrophobic moiety that interact with the WPF shelf (Trp81, Pro82 and Phe83 in BRD4-BD1) and their gatekeeper Ile (Ile146 in BRD4-BD1).



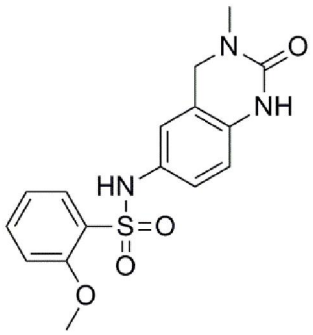
13
I-BET762
IC_{50} BRD4(BD1) = 120 nM



14
JQ1
IC_{50} BRD4(BD1) = 24 nM



15
I-BET151
IC_{50} BRD4(BD1) = 18 nM



16
PFI-1
IC_{50} BRD4(BD1) = 220 nM

Figure 9: First potent BET inhibitors

These inhibitors can be categorized according to their chemical structures into two main groups: inhibitors with triazole or isoxazole moieties and inhibitors with pyridinone moiety. However, new fragments and molecules that were recently developed, have other motifs.

3.1. Inhibitors with triazole or isoxazole moieties:

The Constellation group discovered aminoisoxazole fragment **(17) (Figure 10)** that is capable of forming of the important hydrogen bond with the Asn residue and making a hydrophobic interaction with the gatekeeper Ile residue. Based on fragment**17** besides SAR extracted from compound **13** and **14**, they designed compound **18** (**Figure10**).[53] Moreover, they developed **compound 19 (Figure 10)** with low unbound clearance.[54] Further optimization of these series of compounds has led to a fragment derived inhibitor (**compound 20 Figure 10**).[55]

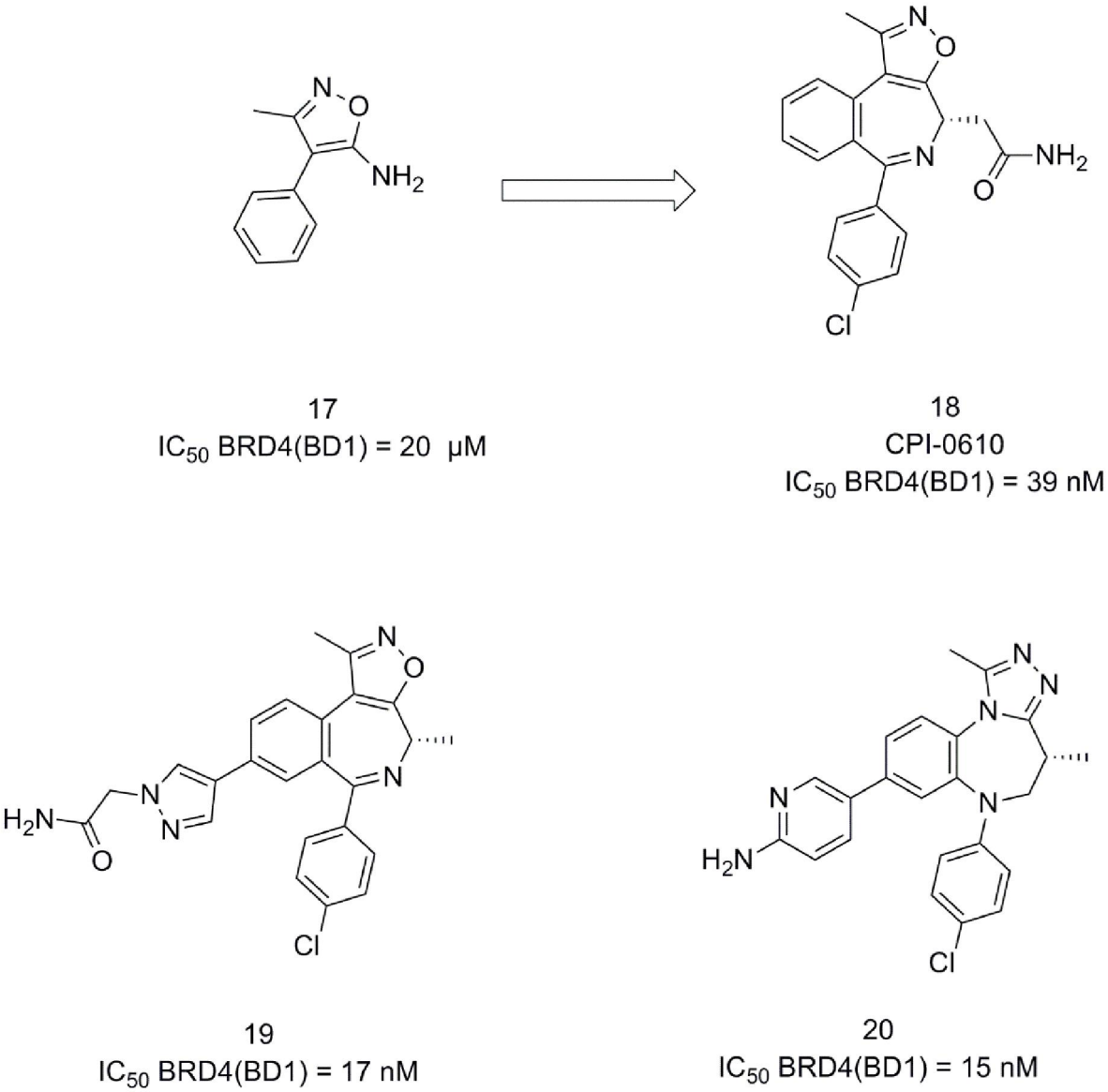
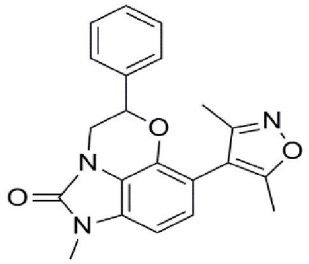


Figure 10: Constellation group designed inhibitors

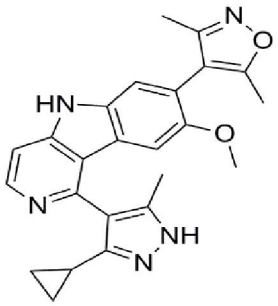
Using I-BET151 (**15**) as their lead, many research groups designed and synthesized new inhibitors with dimethyl isoxazole moiety. Reported scaffolds are for example tricyclic scaffolds (**21**)[56], pyrido [4,3-b] indole (**22**)[57], benzimidazolone (**23**)[58], benzimidazole (**24**)[59], pyrolopyridine (**25**)[59], indolinone (**26**)[60] and Dihydroquinazolinone (**27**)[61] (**Figure11**).

3.2. Inhibitors with pyrdinone moiety:

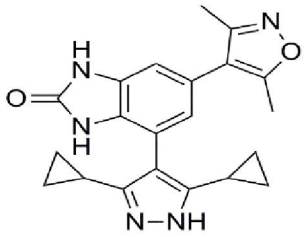
The discovery of PFI-1 (**compound 16** which is the first inhibitor in this class **[Figure 9]**) was developed from a fragment (**28 Figure 12**).[52] Then inhibitors with different heterocycles like furopyridinone (**29**)[62], theinopyridinone (**30**)[63], pyrrolopyridinone (**31**)[64], pyridinone (**32**)[65] and macrocycle (**33 Figure 13**)[66] were developed. Furopyridinone inhibitor (**29 Figure 13**) showed a 100-fold selectivity for BRD4-BD1 over the BRD4-BD2 whereas the pyrrolopyrdinone (**26 Figure 13**) exhibited selectivity for BD2 of BRD4 over BD1. These results reveal the possibility of designing probes for individual bromodomains and exploring the contribution of each BD.[62,63,64]



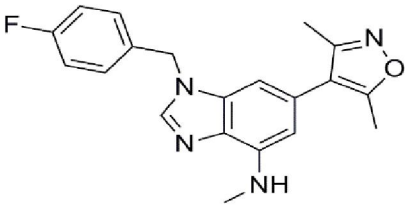
21
IC_{50} BRD4(BD1) <100 nM



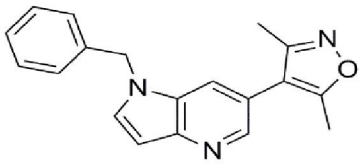
22
IC_{50} BRD4(BD1) = 75.5 nM



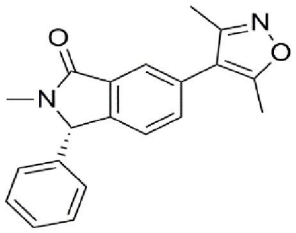
23
IC_{50} BRD4(BD1) = 12.9 nM



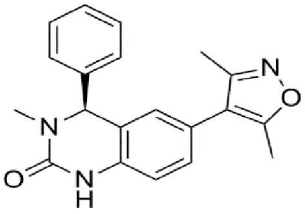
24
IC_{50} BRD4(BD1) <300 nM



25
IC_{50} BRD4(BD1) <300 nM

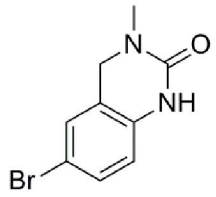


26
IC_{50} BRD4(BD1) = 14 nM

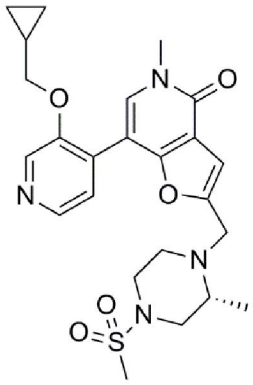


27
IC_{50} BRD4(BD1) = 27 nM

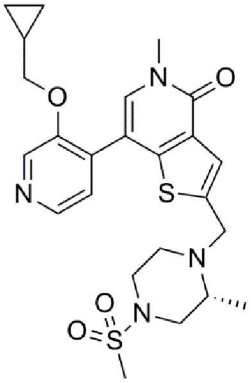
Figure 11: examples for inhibitors with dimethylisoxazole moiety



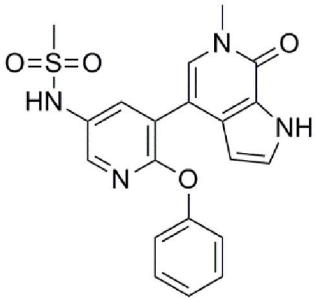
28
IC_{50} BRD4(BD1) = 23 μM



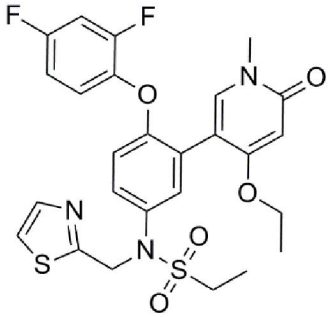
29
IC_{50} BRD4(BD1) <10 nM



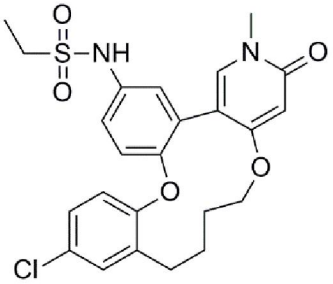
30
IC_{50} BRD4(BD1) <100 nM



31
IC_{50} BRD4(BD1) = 1.2 nM



32
IC_{50} BRD4(BD1) = 110 nM

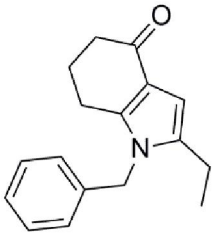


33
IC_{50} BRD4(BD1) = 9 nM

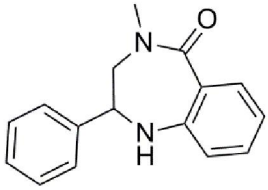
Figure 12: examples for inhibitors with pyridinone moiety

3.3. Other motifs:

A group at Zurich University uses an *in silico* fragment-based screening approach. First, small fragments were obtained by the decomposition of a library of compounds. Fragments with good binding energy were chosen (called anchor fragments). Second, molecules with anchor fragments were used for flexible docking thus resulting molecules with high ligand efficiency (LE). They began with a library of 9 million compounds, only 24 hits were *in vitro* tested to obtain 2 active hits **37** and **38 (Figure 13)**.[67]



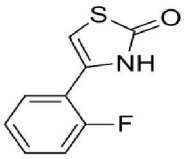
37
IC_{50} BRD4(BD1) = 7 μM
LE = 0.37 Kcal/mol



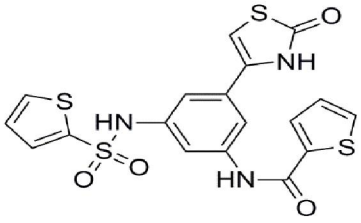
38
IC_{50} BRD4(BD1) = 7.5 μM
LE = 0.37 Kcal/mol

Figure 13

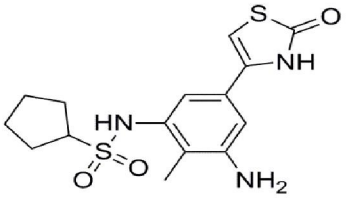
Another research group developed a novel thiazolidinone fragment. The work started by screening a library of fragments using docking, then validation of binding mode of the resulted hits by X-ray crystallography. The fragment was the optimized to obtain two new potent inhibitors **40** and **41 (Figure 14)**.[68]



39
Enzyme inhibition (at 50 μM) = 33%



40
IC_{50} BRD4(BD1) = 230 nM



41
IC_{50} BRD4(BD1) = 140 nM

Figure 14

Recent study utilized a NMR screen to discover two new fragments **46** and **47 (figure 15)** that were optimized in the same work to reach two potent inhibitors **48** and **49 (Figure 15)**.[69]

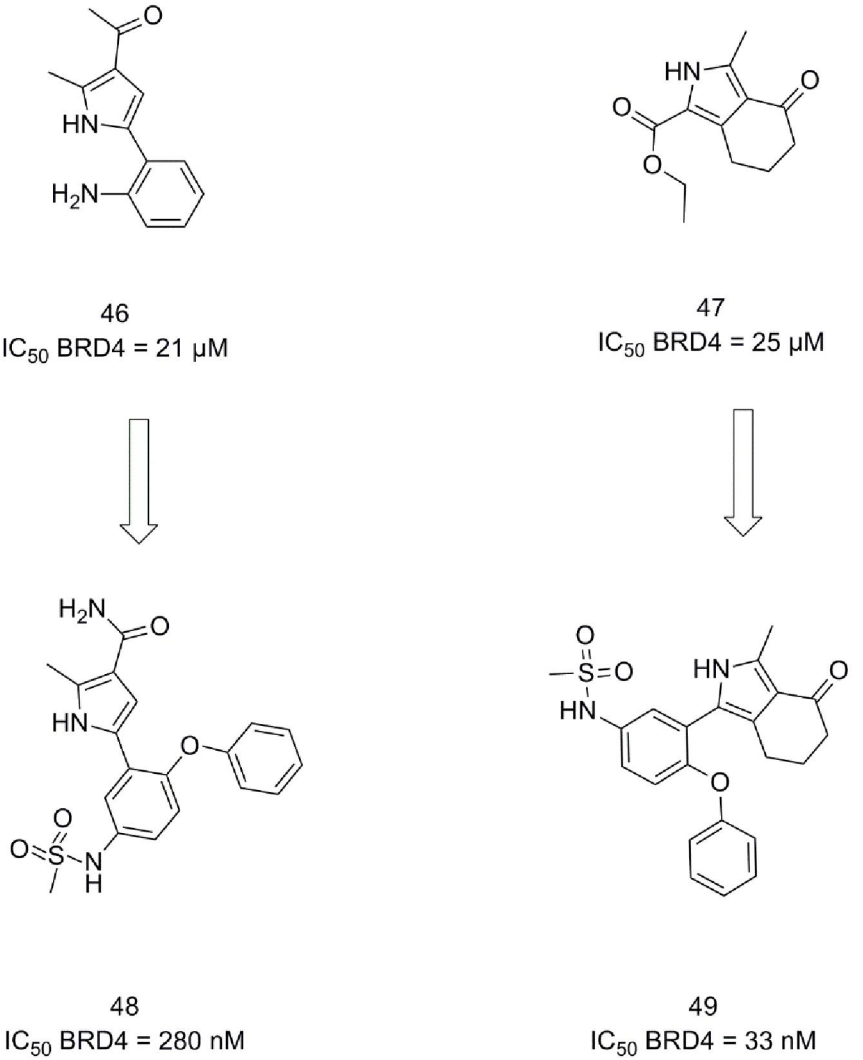
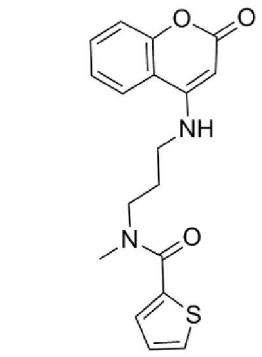


Figure 15

Compound **50** is an example of new inhibitors with novel chromene moiety that is discovered by HTS technique.[70]



50
IC_{50} (BRD4-BD1) = 810 nM

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