Science For A Better Life

Chemical Probe BAY-299

BRPF2 / TAF1(2) probe

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April 6th, 2016

Hit cluster identification

Virtual screening and HTS

1 Virtual screen
BRPF1 and 2 BD

5000-compound library

BRPF2 screen @SGC
180 primary hits

2 HTS of Bayer library
BRPF1 BD

2981 confirmed hits
BRPF1, 2, 3 BD

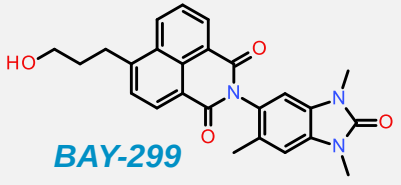
1641 IC_{50} hits
BRPF1, 2, 3 + BRD4 BD

Several overlapping hit clusters

- 7 BRPF2 BD nM hits
- 41 BRPF1 BD nM hits
- No BRPF2 BD selective

- 20 BRPF2 BD nM hits
- 187 BRPF1 BD nM hits
- **1 BRPF2 BD selective**

SAR optimization



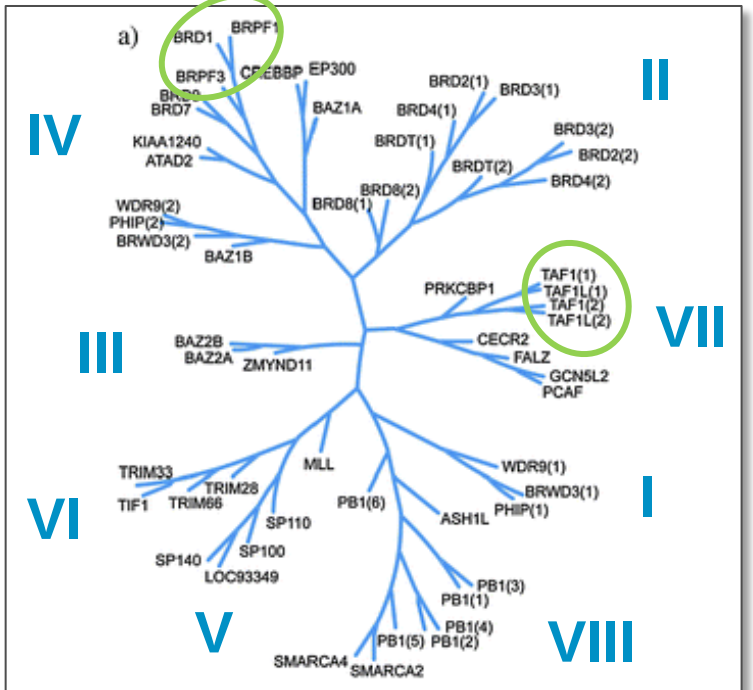
IC_{50} (BRPF1) = 2280 nM
IC_{50} (BRPF2) = 67 nM
IC_{50} (BRD4) = > 20000 nM
IC_{50} (TAF1(2)) = 14 nM

- Dual BRPF2 / TAF1(2) inhibitors identified

- Confidential -

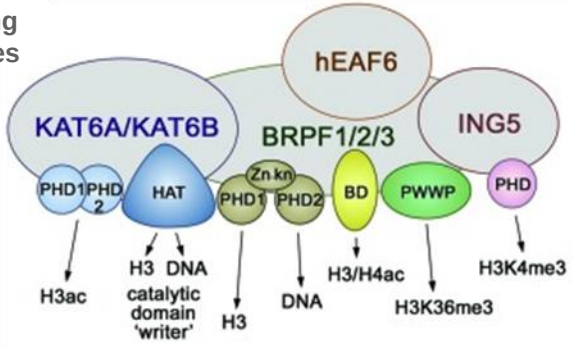
Background

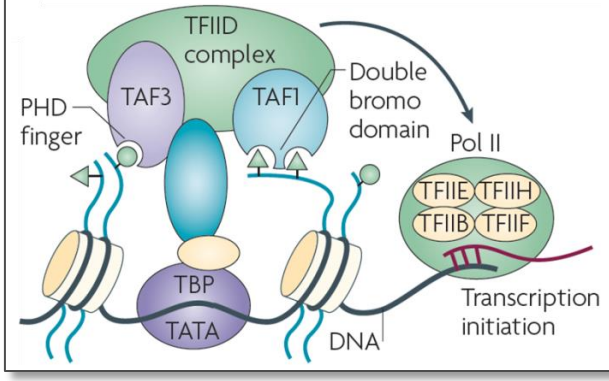
BRPF and TAF1 bromodomain proteins



Phylogenetic tree of human bromodomains
(M. Philpott *et al*., 2011)

BRPFs possess several histone-binding domains and are part of HAT complexes
(B. J. Klein *et al*, 2014)





TAF1 bromodomains interact with acetylated histones and help to stabilize the TFIID complex, thus promoting transcription initiation
(P. Chi *et al*, 2010)

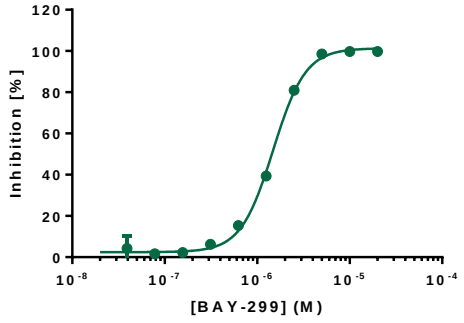
- BRPF proteins interact with DNA and histones, and form complexes with HATs
- TAF1/TAF1L proteins are very similar and part of transcription factor IID complex which controls transcription initiation

- Confidential -

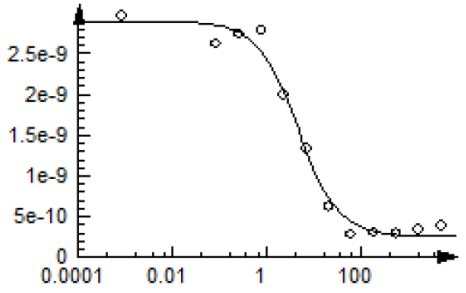
Characterization of BAY-299

BRPF2, TAF1 and TAF1L inhibition

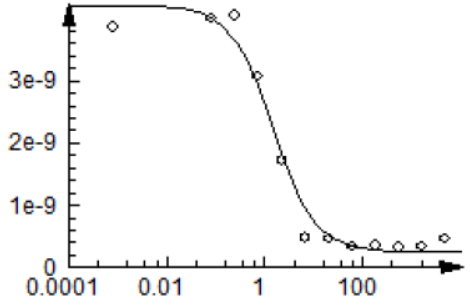
AlphaScreen @ SGC
IC_{50} (BRPF2) = 63 nM



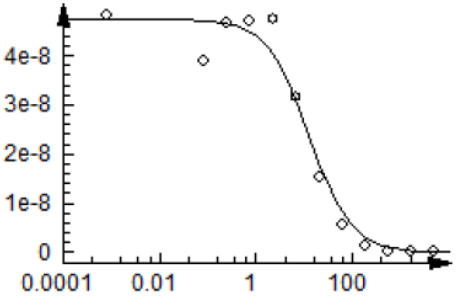
BromoScan @ DiscoverX
K_D (BRPF2) = 6 nM



BromoScan @ DiscoverX
K_D (TAF1) = 2 nM



BromoScan @ DiscoverX
K_D (TAF1L) = 13 nM



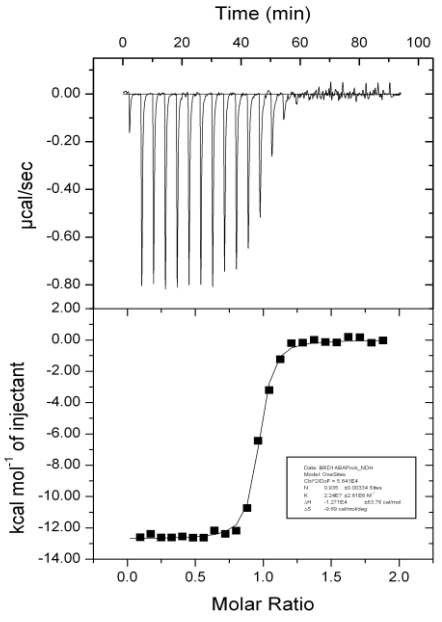
- BAY-299 is a potent BRPF2, TAF1(2) and TAF1L(2) bromodomain inhibitor
- Activity was shown using different biochemical assays

- Confidential -

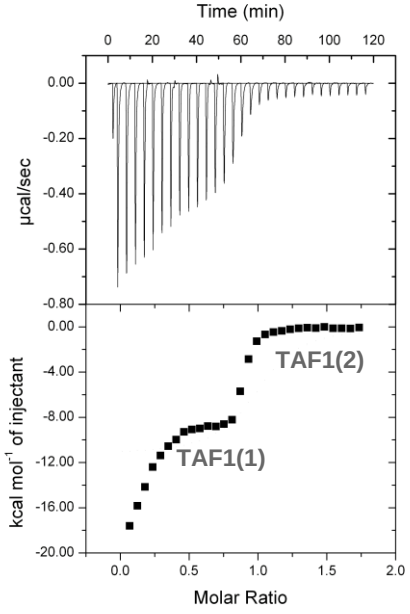
Characterization of BAY-299

BRPF2 and TAF1 binding

ITC @ SGC
K_D (BRPF2) = 45 nM

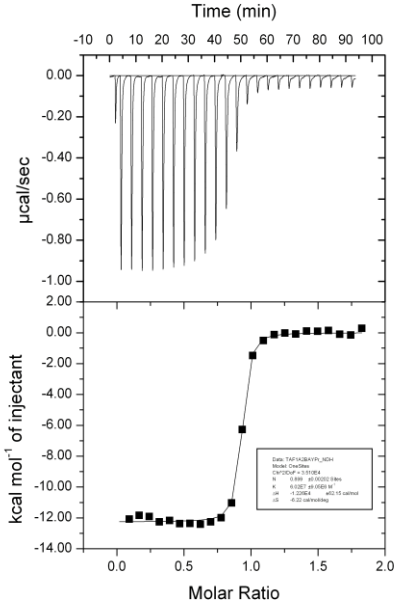


ITC @ SGC
K_D TAF1(1)+(2) not determined



Sequential binding mode

ITC @ SGC
K_D TAF1(2) = 17 nM



- BAY-299 binds to BRPF2 and TAF1(2) with high affinity

- Confidential -

Investigation of binding mode (X-ray)

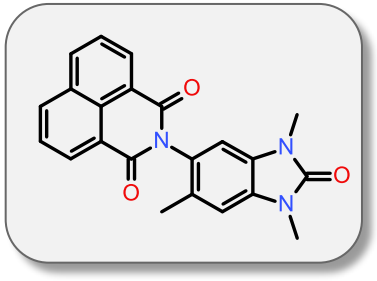
BRPF2 complex with close congener BAY-078

Selectivity Handle

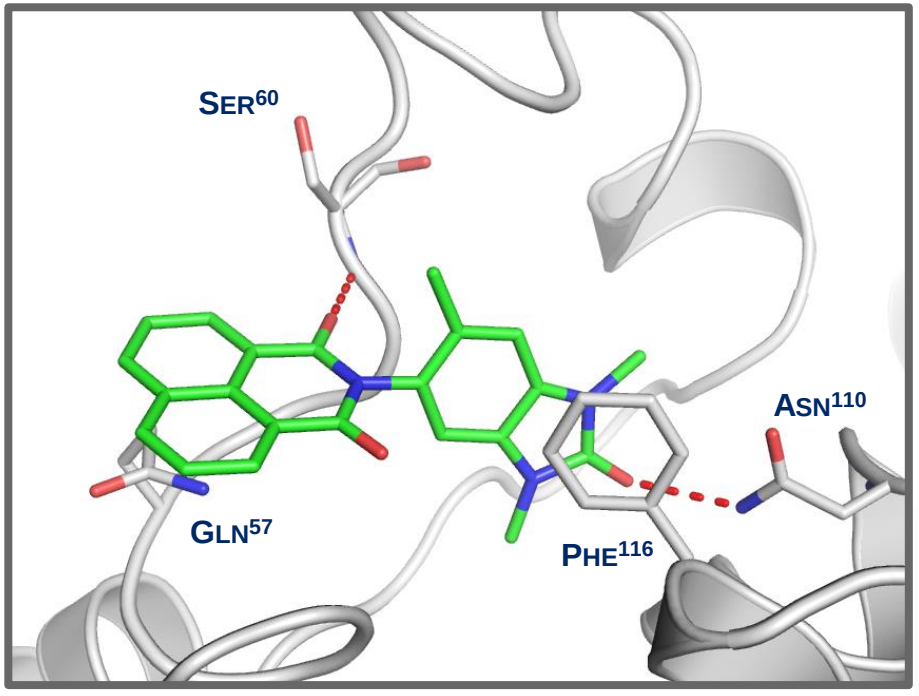
- H-bond between cmpd C=O & protein backbone (SER^{60})
- Similar H-bond in BRPF1 not possible (BRPF1 has a proline at this position) → selectivity

Induced Fit:

- Glutamine (GLN^{57}) sidechain conformation "flips" to interact with tricyclic ring system

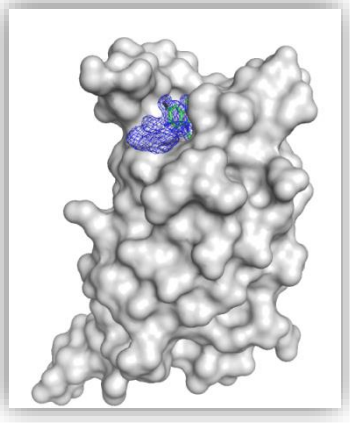


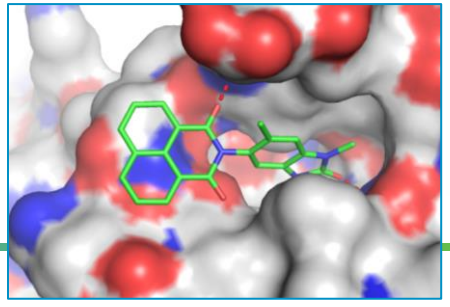
BAY-078



Core

- Methyl induces favorable twist in cmpd required for binding
- H-bond between headgroup and Asparagine (ASN^{110})





- 4 key interactions seen for BAY-078, likely to be similar for BAY-299
- X-ray with BAY-299 ongoing

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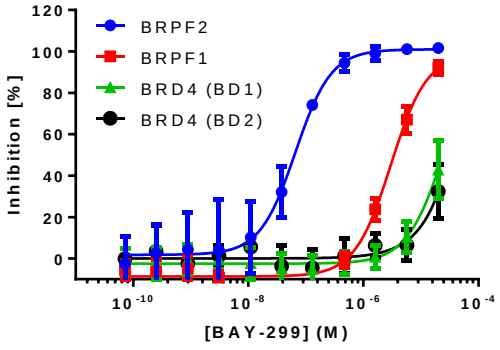
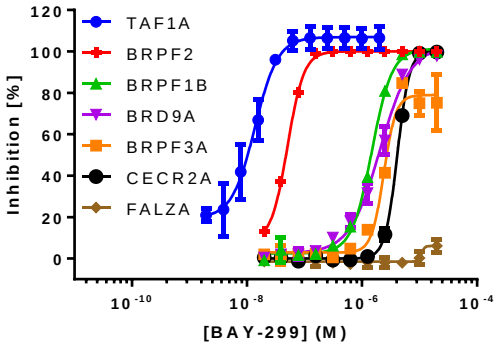
Selectivity over other bromodomains

AlphaScreen and TR-FRET

TR-FRET @ Bayer



Alpha Screen and TR-FRET @ SGC



IC_{50} (BRPF2) = 63 nM*
IC_{50} (TAF1(2)) = 14 nM*

IC_{50} (BRPF1) = 2280 nM (36 fold)
IC_{50} (BRPF3) = 2450 nM (39 fold)

IC_{50} (BRD9) = 2125 nM (34 fold)*
IC_{50} (ATAD2) = >20000 nM (>300 fold)*

IC_{50} (CREBBPA) = 1710 nM (27 fold)
IC_{50} (CECR2) = 4660 nM (74 fold)
IC_{50} (BRD4) = >20000 nM (>300 fold)*

* determined by TR-FRET

- **BAY-299 is selective over other bromodomains**
  - >30-fold selective over the other members of the BRPF family
  - >30-fold selective over close neighbors BRD9 and ATAD2
  - >300-fold selective over BRD4

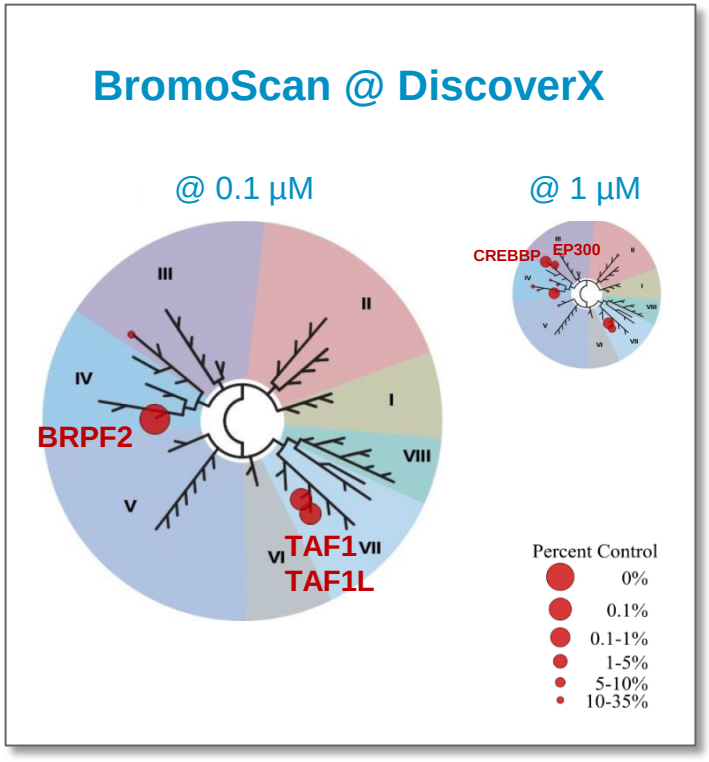
Selectivity over other bromodomains

Thermal Shift Assay and BromoScan

TSA Bromopanel @ SGC (@ 10 µM)

	protein	ΔTm	stdev				
1	protein	ΔTm	stdev	26	FALZ	2,8	1,2
2	ASH1L	1,5	0,5	27	GCN5L2	1,9	0,8
3	ATAD2	2,0	0,5	28	ATAD2B	1,5	0,5
4	BAZ1A	0,8	0,5	29	SP140L	2,1	1,4
5	BAZ1B	0,6	0,5	30	MLL	0,7	0,3
6	BAZ2A	1,5	0,4	31	PB1(1)	1,1	0,3
7	BAZ2B	1,6	0,4	32	PB1(2)	0,1	0,1
8	BRPF2	8,5	0,1	33	PB1(3)	0,5	0,3
9	BRD2(1)	3,2	0,3	34	PB1(4)	1,2	0,3
10	BRD2(2)	1,3	0,3	35	PB1(5)	1,0	0,1
11	BRD3(1)	3,1	1,0	36	PB1(6)	0,8	0,2
12	BRD3(2)	1,7	0,6	37	PCAF	1,5	0,3
13	BRD4(1)	2,4	0,7	38	PHIP(2)	1,1	0,8
14	BRD4(2)	3,4	0,8	39	SMARCA2	0,3	0,1
15	BRD7	2,8	0,3	40	SMARCA4	0,9	0,4
16	BRD9	1,6	0,1	41	SP140	1,7	1,0
17	BRDT(1)	0,9	0,3	42	TAF1(2)	3,6	0,2
18	BRDT(2)	1,8	0,5	43	TAF1(1)	1,4	0,3
19	BRPF1A	0,8	0,3	44	TAF1L(1)	1,4	0,3
20	BRPF1B	2,4	0,3	45	TAF1L(2)	4,8	0,6
21	BRPF3	3,4	0,5	46	TIF1-bromo	1,4	0,5
22	BRWD3(2)	1,4	0,5	47	TIF1-phd-bromo	0,6	0,3
23	CECR2	3,2	0,4	48	TRIM28	0,6	0,7
24	CREBBP	4,6	0,2	49	WDR9(2)	0,7	0,5
25	EP300	4,8	0,4				

BromoScan @ DiscoverX



- Selectivity of BAY-299 is confirmed in larger bromodomain panel
- Strongest thermal shift observed for BRPF2
- Additional activity seen for TAF1(2) and TAF1L(2)
- Binding seen for CREBBP not observed in AlphaScreen (previous slide) or ITC (K_D = 1.4 µM)

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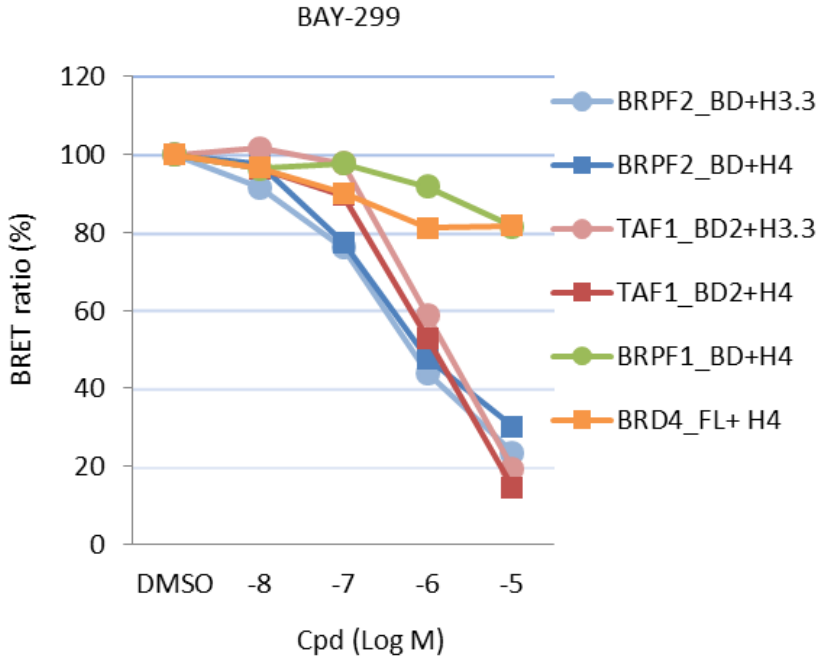
Characterization of BAY-299

Cellular potency: NanoBRET results



	BAY-299 IC_{50} (nM)
BRPF2 BD/H3.3	825
BRPF2 BD/H4	**575**
TAF1 BD2/H3.3	1400
TAF1 BD2/H4	**970**
BRPF1 BD/H4	>10000
BRD4 FL/H4	>10000

- **BAY-299 shows strong cellular activity**
  - Inhibition of BRPF2 BD/H3.3 and H4 interaction
  - Inhibition of TAF1 BD2/H3.3 and H4 interaction
  - ➢ **Disruption of chromatin binding**
- **No inhibition of BRPF1 BD/H4 or BRD4 FL/H4 interaction**

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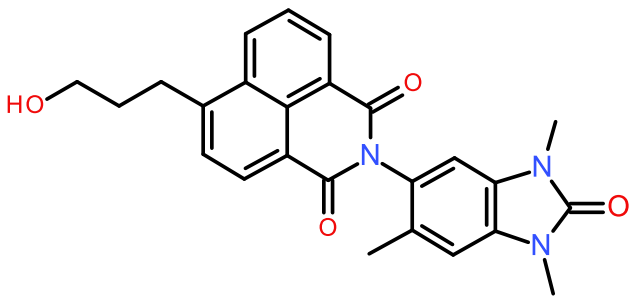
BRPF2 / TAF1(2) probe BAY-299

Overview



BAY-299

Basic profile

IC_{50} BRPF2 (TR-FRET / AlphaScreen)		67 / 63 nM
IC_{50} TAF1(2) (TR-FRET)		14 nM
Selectivity	IC_{50} BRPF1 (TR-FRET / AlphaScreen)	3150 / 2280 nM
	IC_{50} BRPF3 (AlphaScreen)	2450 nM
	IC_{50} BRD4 (TR-FRET)	>20000 nM
IC_{50} (BRPF2 BD/H4) Cellular assay		575 nM
IC_{50} (TAF1 BD2/H4) Cellular assay		970 nM
GI_{50} Proliferation assay		900 - >10000 nM
Selectivity Kinases (# = 16)		>20000 nM
Selectivity Lead profiling screen (# = 68)		>5000 nM

Lead-like profile

$MW_{corrected}$	429.5 g/mol
TPSA	81 Å²
Measured logD (pH 7.5)	2.0
Calculated logD (pH 7.5)	2.5
Solubility	10 mg/L
Stability in r/h plasma	Stable for at least 4 h at 37 °C

in vitro pharmacokinetic profile

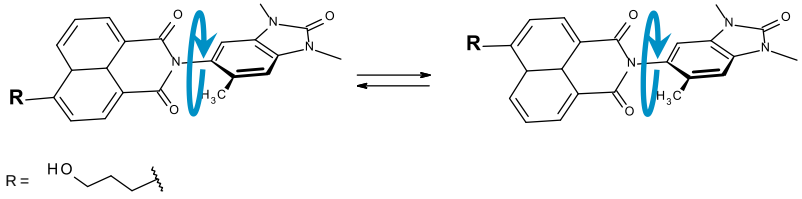
		CL_{int} [L/h/kg]	F_{max} [%]
Hepatocytes	dog / rat	1.5 / 3.8	13 / 9.4
Liver Microsomes	mouse / rat	0.19 / 0.15	96 / 96
	dog / human	<0.001 / <0.001	100 / 100

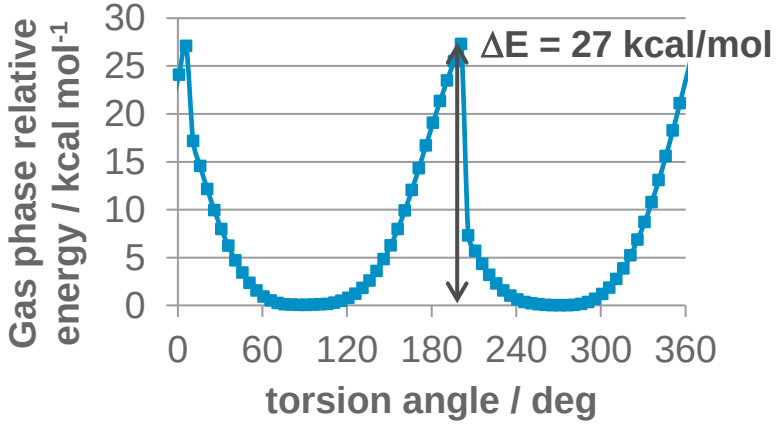
Caco2 permeability	P_{app}(A-B) [nm/s]	P_{app}(B-A) [nm/s]	Ratio
	163	191	1.2

Characterization of probe BAY-299

Atropisomerism

Methyl group induces axial chirality





Relaxed coordinate scan for BAY-299, B3LYP/6-31G**

- **Similar experimental profile**, *e.g.*
  (see full profile in the backup)

	TAF1 IC_{50},HTRF [nM]	BRPF2 IC_{50},HTRF [nM]	BRPF1 IC_{50},HTRF [nM]
Atrop 1	17	62	5860
Atrop 2	11	74	5490

- Calculated barrier height classifies BAY-299 as **class 2 atropisomers** (*J. med. Chem.* **2011**, *54*, 7005-7022): General scientific recommendation **to develop as a mix (racemate)**
- Stirring experiments:
  - at 37 °C in DMSO and CH_3CN showed begin of racemization after 1 week;
  - at 80 °C in DMSO showed 30% racemization after 2 days.

➔ Decision for racemate (BAY-299)

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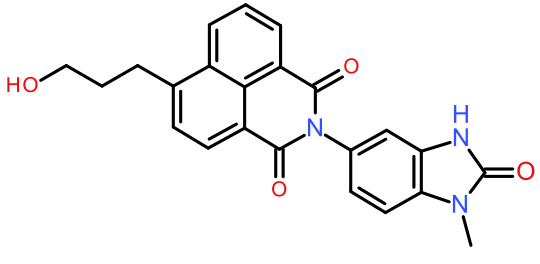
Negative control of BAY-299

Proposed candidate



BAY-364

Basic profile		
IC_{50} BRPF2 (TR-FRET, AlphaScreen)		>20000 nM
IC_{50} TAF1(2) (TR-FRET)		2880 nM
Selectivity	IC_{50} BRPF1 (TR-FRET, AlphaScreen)	>20000 nM
	IC_{50} BRD4 (TR-FRET)	>20000 nM
IC_{50} (BRPF2 BD/H4) Cellular assay		>10000 nM
IC_{50} (TAF1 BD2/H4) Cellular assay		>10000 nM
GI_{50} Proliferation assay		>10000 nM
Selectivity Kinases (# = 14)		>15000 nM

Lead-like properties	
$MW_{corrected}$	401.4 g/mol
TPSA	90 Å²
Measured logD (pH 7.5)	1.7
Calculated logD (pH 7.5)	1.6
Solubility	ongoing

in vitro pharmacokinetic profile				
		CL_{int} [L/h/kg]	F_{max} [%]	
Hepatocytes	rat	3.7	13	
Liver Microsomes	human	0.0042	100	
Caco2 permeability		P_{app}(A-B) [nm/s]	P_{app}(B-A) [nm/s]	Ratio
		25	189	7.4

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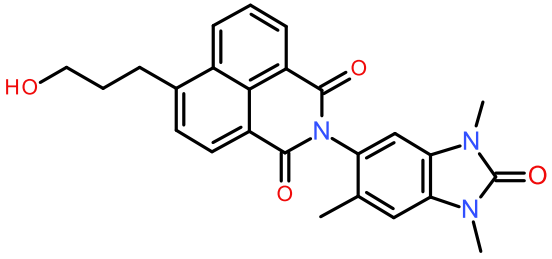
BRPF2 / TAF1 probe BAY-299

Summary



BAY-299

- First potent and cell permeable BRPF2 inhibitor with high selectivity towards BRPF1 (34-fold) and BRPF3 (39-fold)
- BAY-299 shows no activity on BRD4
- Cellular activity for BRPF2 confirmed in NanoBRET
- Additional potent TAF1(2) inhibition, confirmed in NanoBRET
- Bromodomain selectivity tested in TR-FRET, BromoScan, AlphaScreen and TSA
- Selectivity vs. other targets determined in kinase panel (in-house) and Lead Profiling Screen (Eurofins Panlabs)

BAY-299 is proposed as a dual BRPF2 and TAF1(2) probe

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BRPF2 / TAF1 probe BAY-299

Acknowledgements

Amaury Ernesto Fernández-Montalván
Antonius ter Laak
Clara Christ
Detlef Stoeckigt
Oliver Schenk
Simon Holton
Stephan Siegel
Ursula Egner

Andrea Haegebarth
Ingo Hartung
Marcus Bauser

Cora Mund
Marion Hitchcock

Anke Mueller-Fahrnow

Julia Meier
John Porter
Kilian Huber
Oleg Fedorov
Peter Brown
Susanne Müller-Knapp
Tony Tumber

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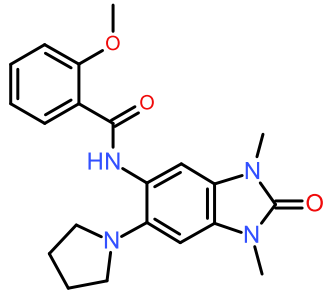
Thank you!

Literature known chemical probes

BRPF inhibitors

BRPF1B inhibitor

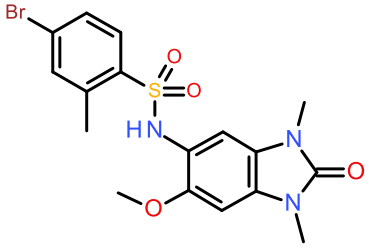


PFI-4

IC_{50} (BRPF1) = 42 nM
IC_{50} (BRPF2) = 869 nM
IC_{50} (BRD4) = 8340 nM

(*Med. Chem. Lett.*, **2014**, *5*, 1190-1195)

BRPF1,2,3 inhibitor

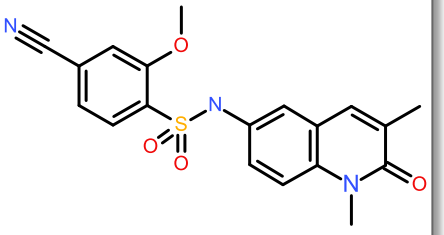


OF-1

IC_{50} (BRPF1) = 85 nM
IC_{50} (BRPF2) = 892 nM
IC_{50} (BRD4) = 9200 nM

(*J. Med.Chem.*, **2016**, *59*, 1642-1647)

BRPF1,2,3 inhibitor

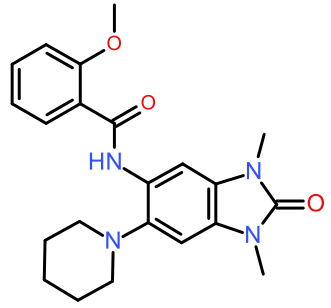


NI-57

IC_{50} (BRPF1) = 25 nM
IC_{50} (BRPF2) = 137 nM
IC_{50} (BRD4) = 6620 nM

(WO 2016/034512 A1)

BRPF1B inhibitor



IC_{50} (BRPF1) = 30 nM
IC_{50} (BRPF2) = 1270 nM
IC_{50} (BRD4) = 9080 nM

(*Med. Chem. Lett.*, **2014**, *5*, 1190-1195)

Chemical probes

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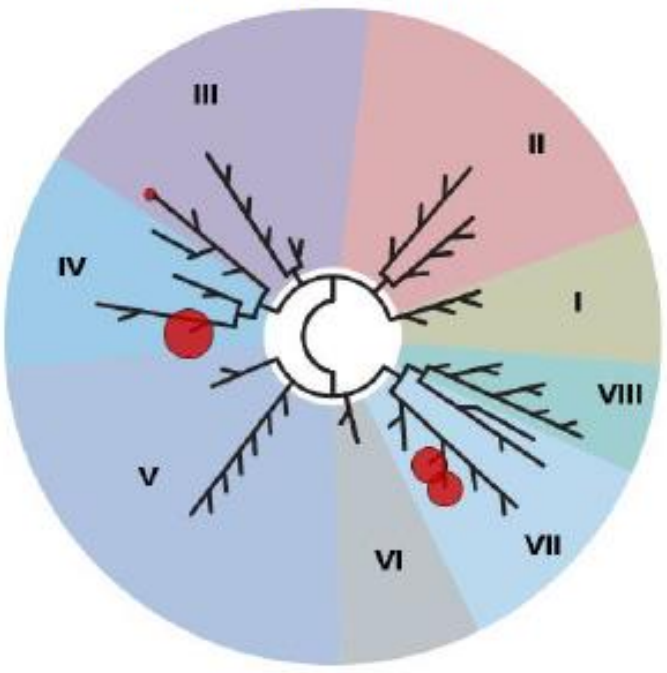
Selectivity over other bromodomains

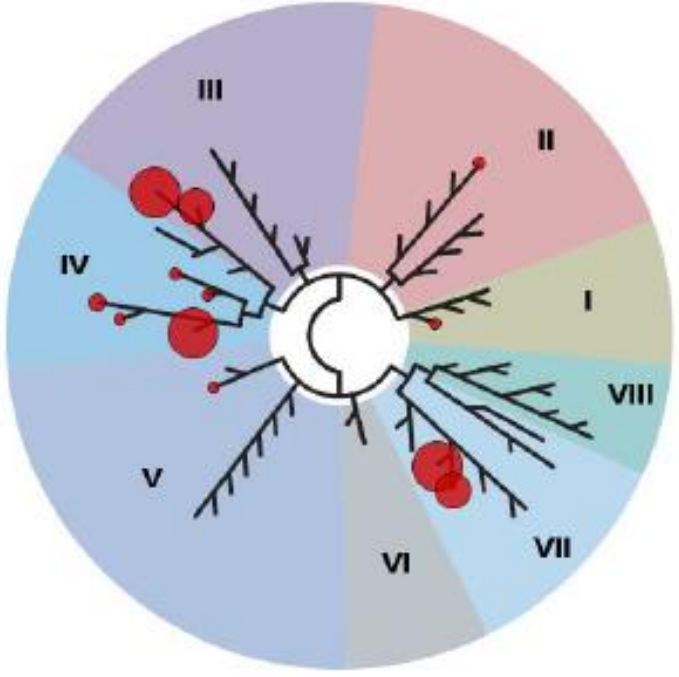
Full BromoScan data for BAY-299

Target	BAY-299	
Gene Symbol	%Ctrl @ 100nM	%Ctrl @ 1000nM
ATAD2A	93	87
ATAD2B	73	59
BAZ2A	53	41
BAZ2B	68	14
BRD1	0	0
BRD2(1)	75	15
BRD2(2)	72	74
BRD3(1)	96	71
BRD3(2)	91	89
BRD4(1)	75	42
BRD4(2)	82	71
BRD7	70	14
BRD9	68	18
BRDT(1)	87	75
BRDT(2)	93	91
BRPF1	79	8.8
BRPF3	97	25
CECR2	74	15
CREBBP	30	0
EP300	41	0.5
FALZ	72	37
GCN5L2	100	69
PBRM1(2)	74	70
PBRM1(5)	81	70
PCAF	88	71
SMARCA2	73	56
SMARCA4	100	100
TAF1(2)	0.8	0.2
TAF1L(2)	0.7	0
TRIM24(PHD,Bromo.)	81	78
TRIM33(PHD,Bromo.)	92	96
WDR9(2)	61	50

BAY-299 @ 0.1 µM



BAY-299 @ 1 µM



- **Similar selectivity profile as observed in SGC's TSA panel**

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Characterization of probe BAY-299

in vitro DMPK of BAY-299

Hepatocytes		CL_{int} [L/h/kg]			F_{max} [%]	
	rat/dog	3.8 / 1.5			9.4 / 13	
Liver Microsomes	mouse/rat	0.19 / 0.15			96 / 96	
	dog/human	<0.001 / <0.001			100 / 100	
Caco2 permeability		P_{app}(A-B) [nm/s]			P_{app}(B-A) [nm/s]	Ratio
		163			191	1.2
CYP inhibition [µM]		1A2	2C8	2C9	2D6	3A4
		>20	>20	>20	>20	>20
CYP induction		PXR flag green; NOEL(1A2) > 10000 µg/L, NOEL(3A4) = 1111 µg/L (hint on CAR-mediated induction)				(no hint on TDI)

- **Metabolic stability of BAY-299 is high in liver microsomes across species, but low in hepatocytes of rat and dog**
- **BAY-299 cell permeability is high with no hint on active transport**
- **CYP inh >20 µM and no hint on time-dependent inhibition**

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Characterization of probe BAY-299

Comparison of the corresponding atropisomers

Basic profile		BAY-299	*Atrop 1*	*Atrop 2*
IC_{50} TAF1 (TR-FRET)		14 nM	17 nM	11 nM
IC_{50} BRPF2 (TR-FRET)		67 nM	62 nM	74 nM
Selectivity	IC_{50} BRPF1 (TR-FRET)	3150 nM	5860 nM	5490 nM
	IC_{50} BRD4 (TR-FRET)	>20000 nM	>20000 nM	>20000 nM
	IC_{50} BRPF3 (AlphaScreen)	2450 nM	2160 nM	4170 nM
	IC_{50} BRD9 (TR-FRET)	>4250 nM	5040 nM	2190 nM
K_D (BRPF2) ITC		45 nM	36 nM	37 nM
IC_{50} (BRPF2) Cellular mechanistic assay		500 nM	354 nM	565 nM
CaCo2 Papp(A-B) / Papp(B-A) [nm/s] / ratio		163 / 191 / 1.2	181 / 183 / 1.0	151 / 206 / 1.4

- **Similar profiles for BAY-299 (racemate) and individual atropisomers**

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