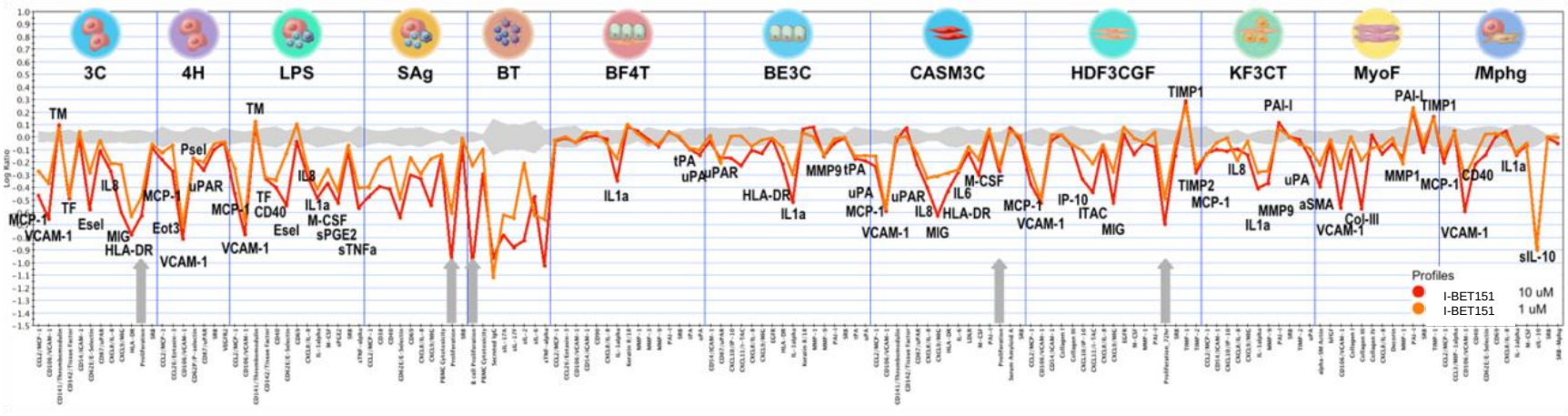
Supplementary Figure 1 - BioMap profile of the BET inhibitor I-BET151

BioMAP profiles of I-BET151 generated using the Diversity Plus™ Panel (http://biomapsystems.com/services/diversity-plus-panel). The compound was tested at 10 (red) and 1 µM (orange). The biomarker readouts measured are indicated along the x-axis. The y-axis shows the log10 expression ratios of the readout level measurements relative to solvent (DMSO buffer) controls. Each data point represents a single well. The gray area above and below the dashed line indicates the 95% significance envelope of DMSO negative controls.

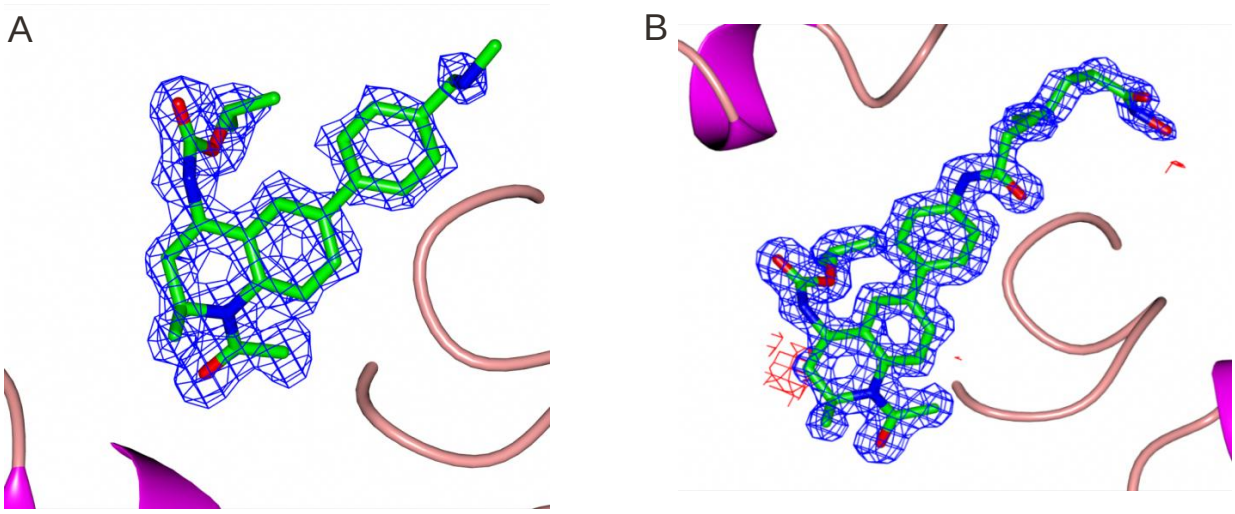


Supplementary Table 1- DiscoveRx BROMOscan™ Profile of I-BET726: Kd values

TARGET		Kd (nM)
ATAD2A	>	30000
ATAD2B	>	30000
BAZ2A	>	30000
BAZ2B	>	30000
BRD1	>	30000
BRD2(1)	=	3.1
BRD2(2)	=	0.66
BRD3(1)	=	1.9
BRD3(2)	=	0.41
BRD4(1)	=	3.5
BRD4(1,2)	=	0.26
BRD4(2)	=	0.4
BRD4(full-length,short-iso.)	=	1.3
BRD7	>	30000
BRD9	>	30000
BRDT(1)	=	3.8
BRDT(2)	=	1
BRPF1	>	30000
BRPF3	>	30000
CECR2	>	30000
CREBBP	=	330
EP300	=	610
FALZ	>	30000
GCN5L2	>	30000
PBRM1(2)	=	7800
PBRM1(5)	>	30000
PCAF	>	30000
SMARCA2	>	30000
TAF1(2)	=	9100
TAF1L(2)	=	16000
TRIM24(Bromo.)	=	9800
TRIM24(PHD,Bromo.)	=	16000
TRIM33(PHD,Bromo.)	>	30000
WDR9(2)	=	20000

Supplementary Figure 2 – X-ray Difference Density for BET Inhibitors: (A) BRD4-BD1/I-BET295 (4CLB.pdb) and (B) BRD4-BD1/DUAL946 (4CL9.pdb)

OMIT difference map (fo-fc) contoured at +3sigma (blue), -3sigma (red)



Supplementary Table 2: X-ray data collection and refinement statistics (Molecular replacement)

(collection on a single crystal)	**BRD4-BD1/I-BET295**	**BRD4-BD1/DUAL946**
Data collection		
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a, *b*, *c* (Å)	41.78, 49.42, 57.27	38.61,43.14,80.17
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Resolution (Å)	57.27-1.60 (1.69-1.60) *	40.08-1.42 (1.48-1.40) *
R_{merge}	0.022(0.085)	0.080(0.742)
$I/\sigma I$	39.5 (11.2)	12.3 (2.7)
Completeness (%)	96.8 (81.5)	99.9 (99.9)
Redundancy	4.3 (2.6)	6.1 (6.1)
Refinement		
Resolution (Å)	37.41-1.60 (1.69-1.60)	40.08-1.42 (1.48-1.40)
No. reflections	66264 (4767)	166241 (23883)
No. uniq reflections	15575 (1851)	27078(3908)
$R_{work/}$ R_{free}	0.165/0.205	0.188/0.199
No. atoms	1408	
Protein	1091	1062
Ligand/ion	30/4	40/4
Water	283	231
B-factors		
Protein	10.88	17.31
Ligand/ion	12.83/10.42	13.81
Water	23.31	32.40
R.m.s deviations		
Bond lengths (Å)	0.0049	0.0058
Bond angles (°)	1.032	1.145

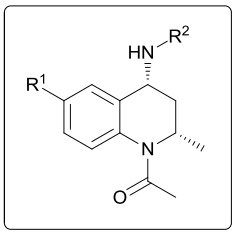
*Highest resolution shell is shown in parenthesis.

Supplementary Figure 3 – Sequence Homology of the BET Family Proteins

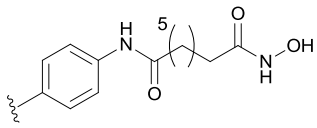
```
                                |~~~ ~~~~αZ~~~~~~|      _____      ZA loop      _____|~~αA
BRD2_BD1     61  PPPPEVSNPK KPGRVTNQLQ YLHKVVMKAL WK---HQFAW PFRQPVDAVK LGLPDYHKII KQPMDMGTIK
BRD3_BD1     21  PPPPEVSNPS KPGRKTNQLQ YMQNVVVKTL WK---HQFAW PFYQPVDAIK LNLPDYHKII KNPMDMGTIK
BRD4_BD1     45  PPPPETSNPN KPKRQTNQLQ YLLRVVLKTL WK---HQFAW PFQQPVDAVK LNLPDYYKII KTPMDMGTIK
BRDT_BD1     14  PPPPEYINTK KNGRLTNQLQ YLQKVVLKDL WK---HSFSW PFQRPVDAVK LKLPDYYTII KNPMDLNTIK
BRD2_BD2    339  --------QS SKKGKLSEQL KHCNGILKEL LSKKHAAYAW PFYKPVDASA LGLHDYHDII KHPMDLSTVK
BRD3_BD2    301  --------HA GKKGKLSEHL RYCDSILREM LSKKHAAYAW PFYKPVDAEA LELHDYHDII KHPMDLSTVK
BRD4_BD2    343  --------AP EKSSKVSEQL KCCSGILKEM FAKKHAAYAW PFYKPVDVEA LGLHDYCDII KHPMDMSTIK
BRDT_BD2    262  --------NV VKTVKVTEQL RHCSEILKEM LAKKHFSYAW PFYNPVDVNA LGLHNYYDVV KNPMDLGTIK

             αA~~|      |~~~~~~~~αB ~~~~~~| BC loop |~~ ~~~~~~αC~~ ~~~~~|
BRD2_BD1    128  RRLENNYYWA ASECMQDFNT MFTNCYIYNK P----TDDIV LMAQTLEKIF LQKVASMPQE
BRD3_BD1     88  KRLENNYYWS ASECMQDFNT MFTNCYIYNK P----TDDIV LMAQALEKIF LQKVAQMPQE
BRD4_BD1    112  KRLENNYYWN AQECIQDFNT MFTNCYIYNK P----GDDIV LMAEALEKLF LQKINELPTE
BRDT_BD1     81  KRLENKYYAK ASECIEDFNT MFSNCYLYNK P----GDDIV LMAQALEKLF MQKLSQMPQE
BRD2_BD2    401  RKMENRDYRD AQEFAADVRL MFSNCYKYNP P----DHDVV AMARKLQDVF EFRYAKMPDE
BRD3_BD2    363  RKMDGREYPD AQGFAADVRL MFSNCYKYNP P----DHEVV AMARKLQDVF EMRFAKMPDE
BRD4_BD2    405  SKLEAREYRD AQEFGADVRL MFSNCYKYNP P----DHEVV AMARKLQDVF EMRFAKMPDE
BRDT_BD2    324  EKMDNQEYKD AYKFAADVRL MFMNCYKYNP P----DHEVV TMARMLQDVF ETHFSKIPIE
```

Supplementary Table 3 – Calculated and Measured Chromatographic logD (pH = 7.4) and PFI Values[1]



A =



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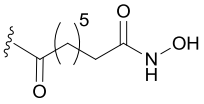
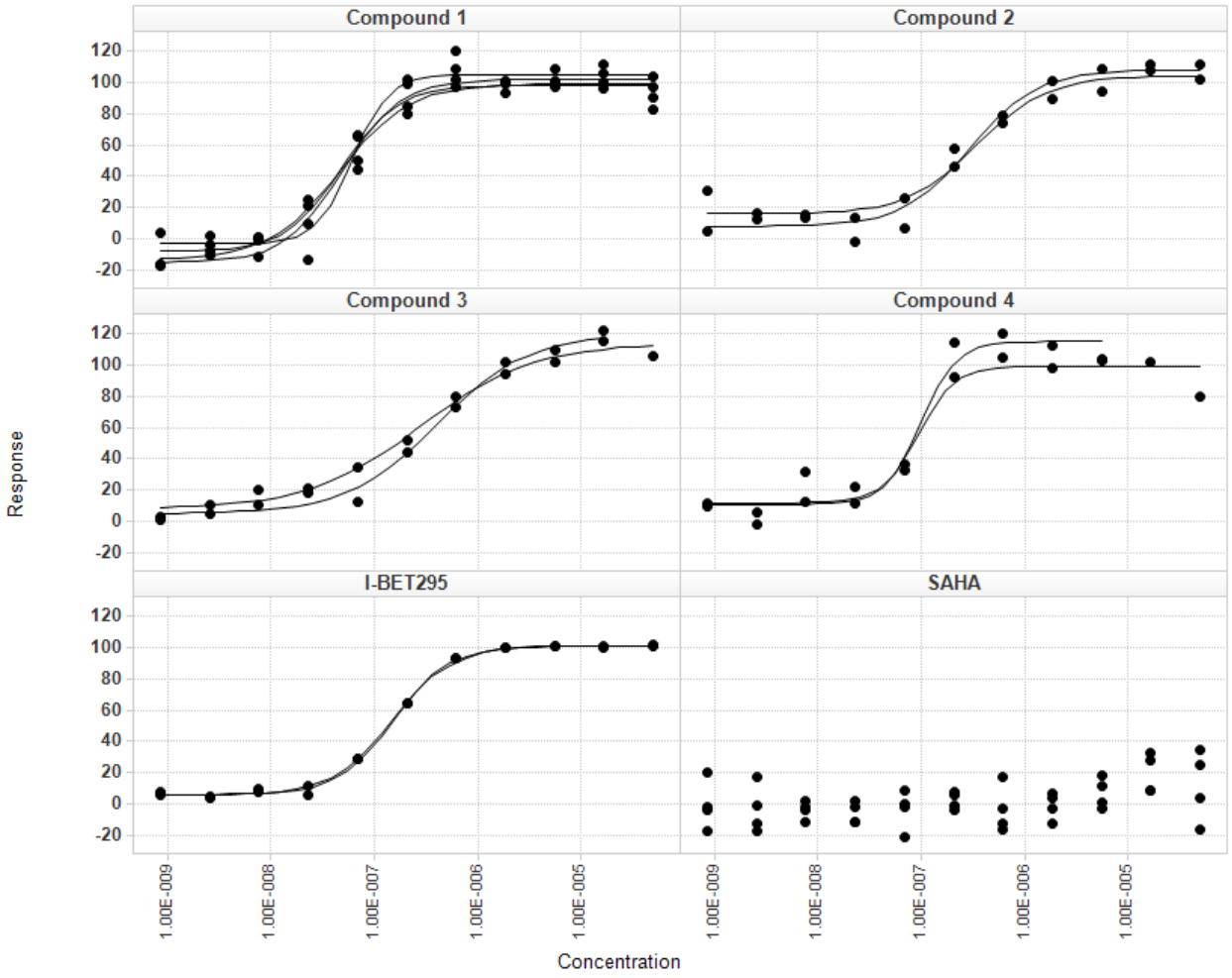
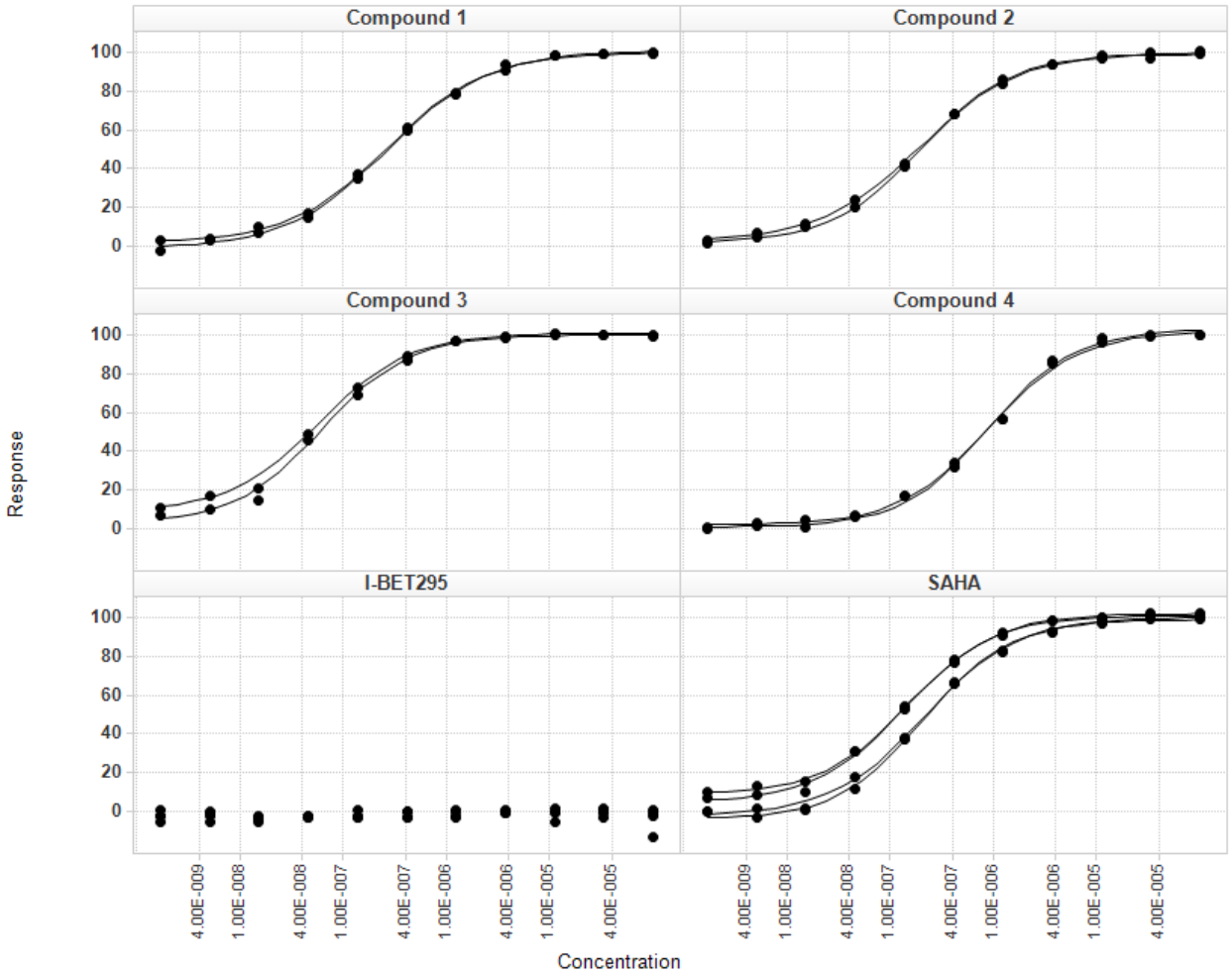


Compound			Calculated			Measured	
	R^1	R^2	Ar ring	cChrom logD pH 7.4	cPFI	mChrom logD pH 7.4	mPFI
1	A	$CO_2{}^iPr$	2	3.3	5.3	3.2	5.2
2	HN-B	$CO_2{}^iPr$	1	2.1	3.1	2.1	3.1
3	Ph	B	2	3.3	5.3	3.1	5.1
4	A	Ph	3	4.0	7.0	4.1	7.1
	I-BET295		2	2.5	4.5	2.1	4.1
	SAHA		1	2.3	3.3	1.8	2.8

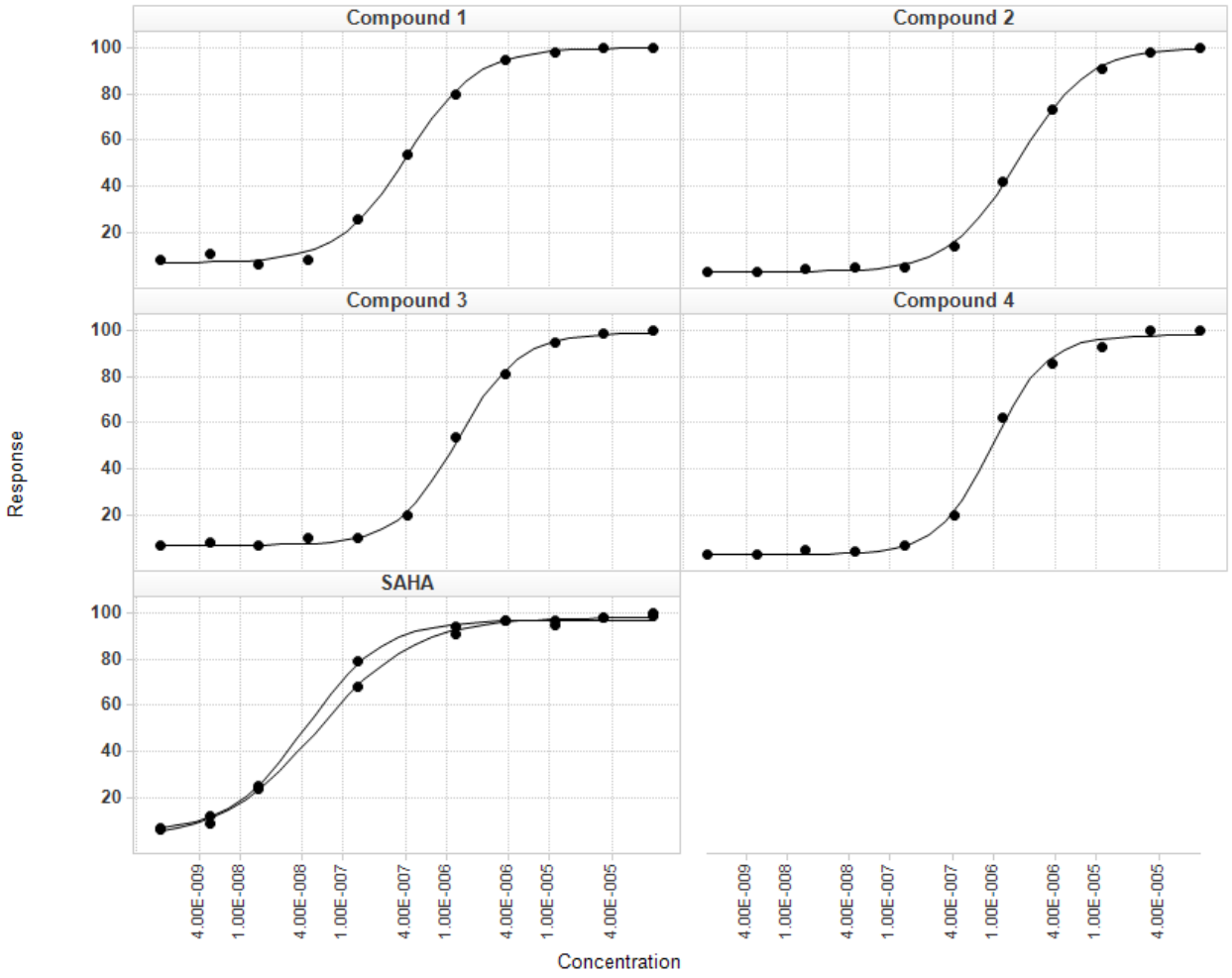
Supplementary Figure 4A – BRD4 HTRF Biochemical Assay - IC_{50} curves for Compounds 1-4, I-BET295 and SAHA



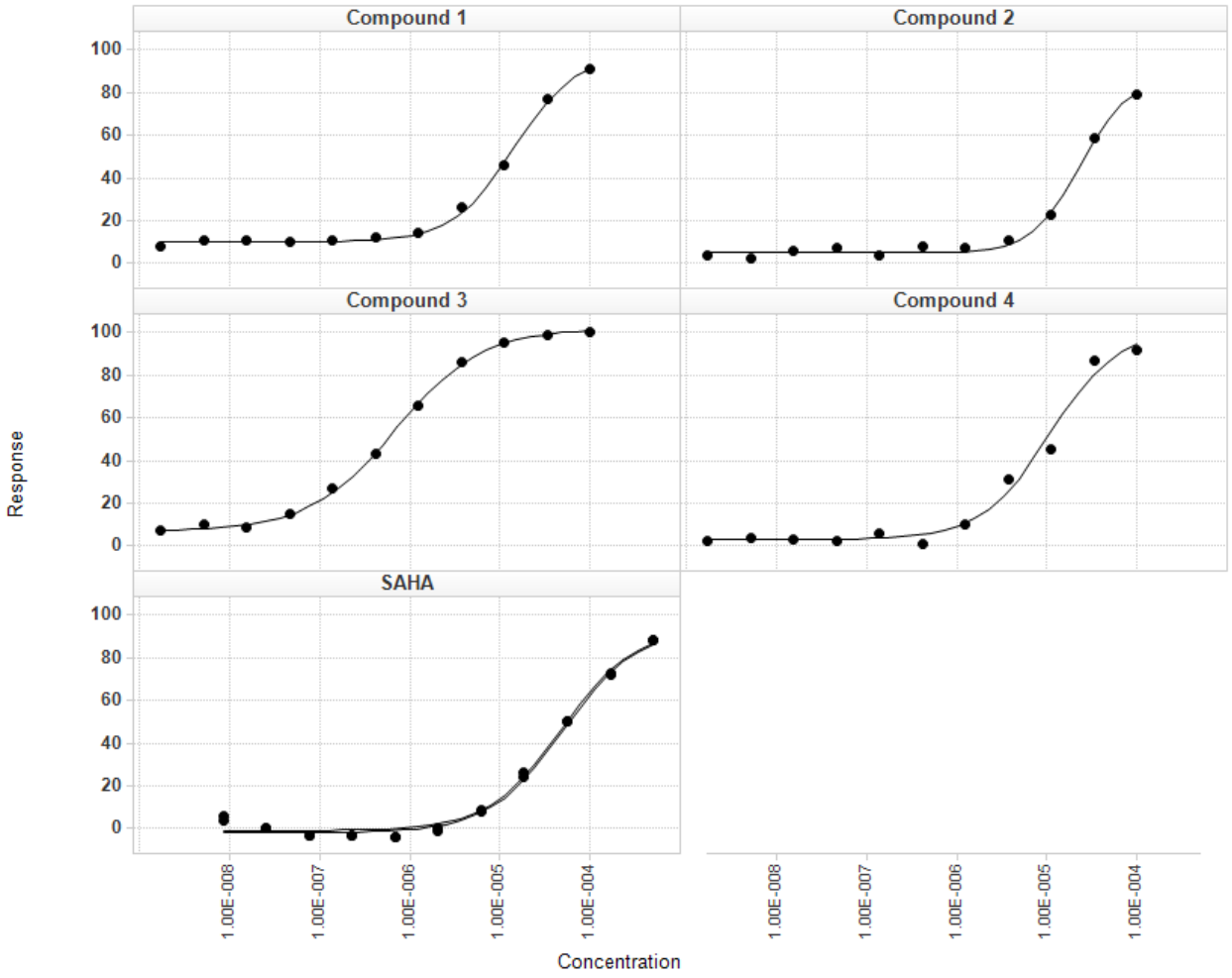
Supplementary Figure 4B – HDAC 1 Biochemical Assay - IC_{50} curves for Compounds 1-4, I-BET295 and SAHA



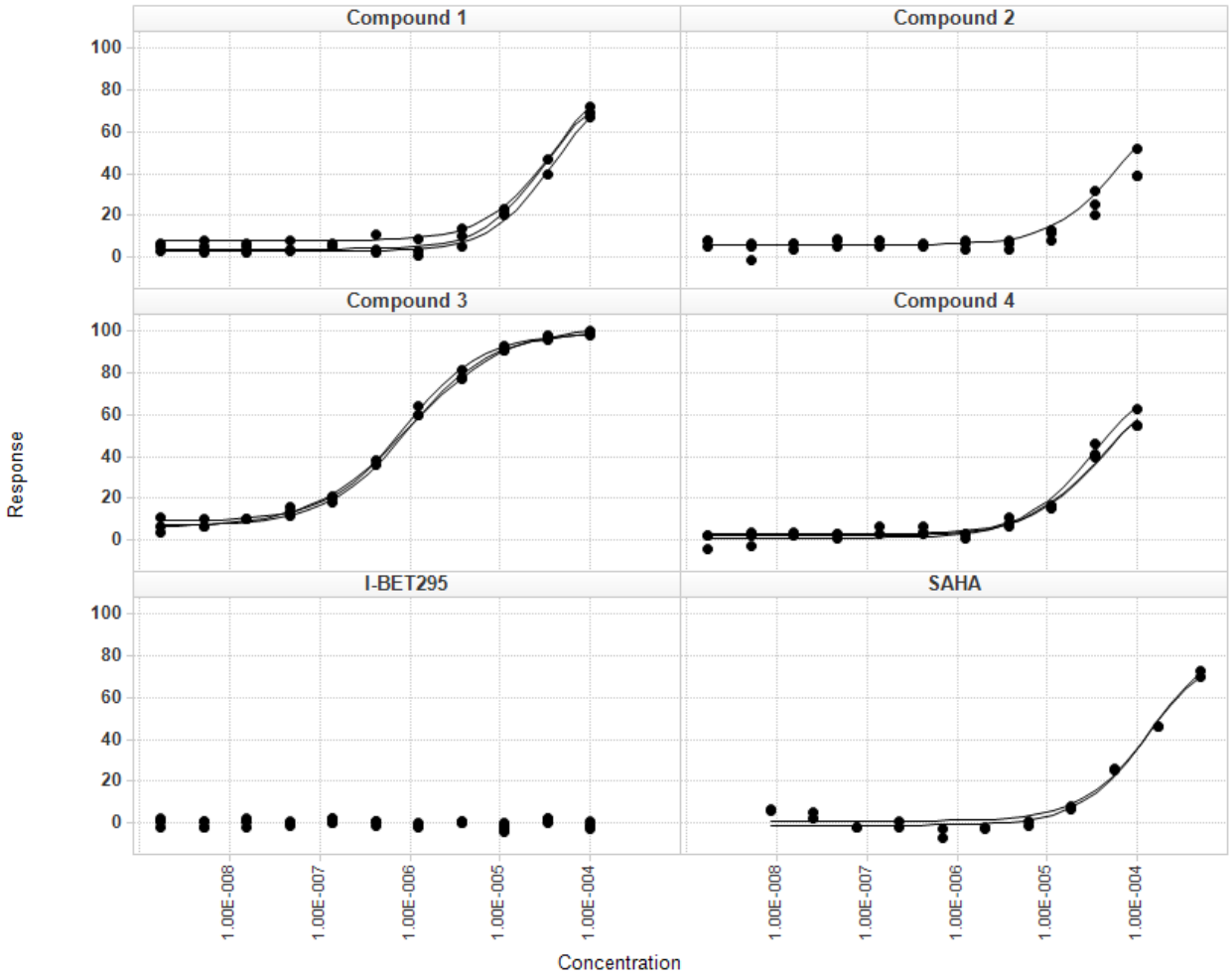
Supplementary Figure 4C – HDAC 6 Biochemical Assay - IC_{50} curves for Compounds 1-4 and SAHA



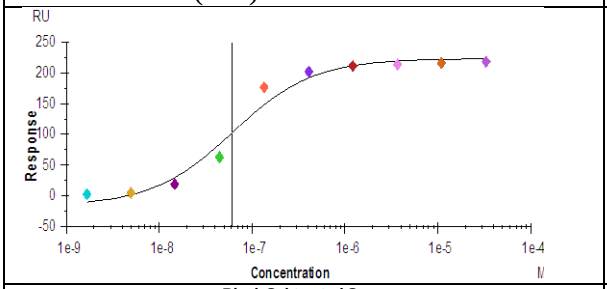
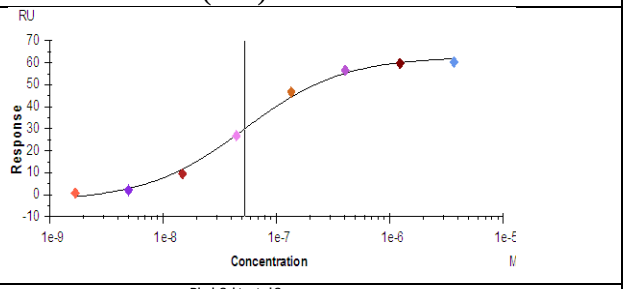
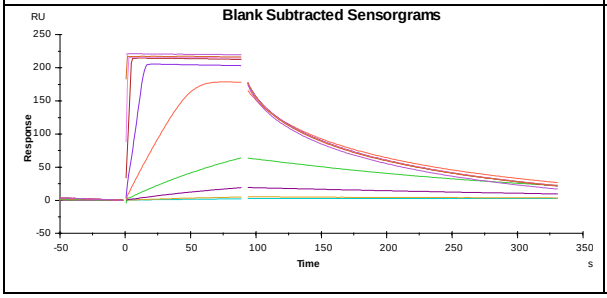
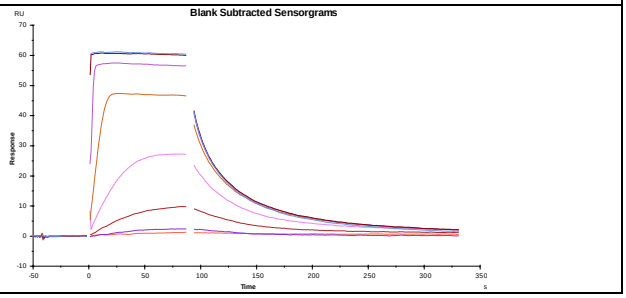
Supplementary Figure 4D – HDAC 7 Biochemical Assay - IC_{50} curves for Compounds 1-4 and SAHA



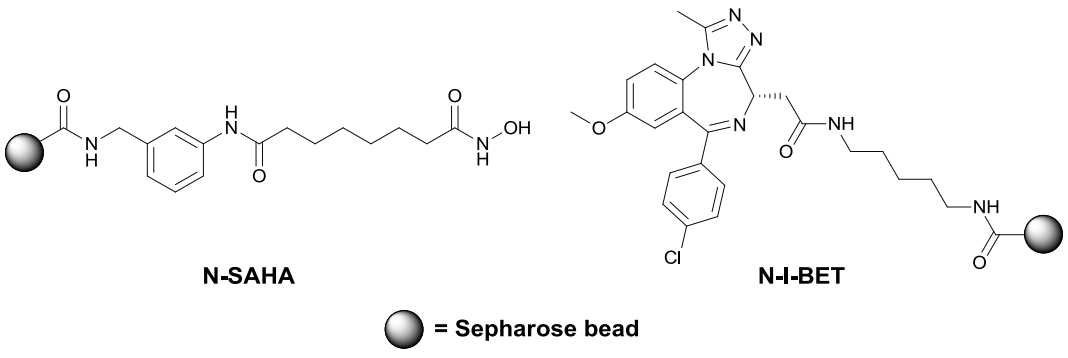
Supplementary Figure 4E – HDAC 9 Biochemical Assay - IC_{50} curves for Compounds 1-4, I-BET295 and SAHA



Supplementary Table 4: Surface Plasmon Resonance binding parameters for DUAL946 and example sensorgram

BRD4 (1-477) Kd = 57±7nM (n=2)	BRD2 (338-437) Kd = 53±0.6nM (n=2)
	
	

Supplementary Figure 5: Structures of N-SAHA and N-I-BET



Supplementary Table 5A: Competition binding experiments of indicated compounds at 10 µM using the N-SAHA matrix

SSM quant: quantified sequence-to-spectrum matches; pep quant: distinct peptides for quantification

					Fold-change relative to vehicle control				
ACCESSION	PROTEIN	ANNOTATION	SSM quant	pep quant	3	I-BET295	DUAL946	19	SAHA
IPI00002922	TBL1XR1	NCOR	28	6	0.06	0.9	0.16	0.92	0.04
IPI00005492	WDR5		10	3	0.14	0.83	0.3	0.89	0.2
IPI00005711	HDAC6		54	12	0.16	0.87	0.1	0.9	0.03
IPI00008531	RCOR1	CoREST	52	14	0.02	0.92	0.21	0.94	0.01
IPI00010239	AMZ2		17	6	0.67	0.93	0.8	1	0.65
IPI00012301	GPS2	NCOR	6	2	0.2	0.95	0.18	0.92	0.17
IPI00012439	HDAC10		41	9	0.5	0.85	0.28	0.89	0.23
IPI00012833	PPP4C		14	3	0.62	0.96	0.93	1.07	0.76
IPI00013004	PDXK		15	5	0.92	1.12	1.1	1.13	1.09
IPI00013774	HDAC1		53	11	0.11	0.81	0.43	0.89	0.12
IPI00018924	HMG20A	CoREST	42	10	0.09	1.08	0.33	1.06	0.09
IPI00022019	SAP30	SIN3	6	2	0.13	0.9	0.18	0.95	0.21
IPI00022810	CTSC		7	3	1.14	1	1.11	1.31	1.13
IPI00022904	PPP4R2		3	2	1.03	1.24	1.13	1.27	1.3
IPI00024913	C21ORF33		9	4	0.89	0.98	1.02	0.98	0.88
IPI00027809	PPP3CB		7	4	0.71	0.81	0.6	0.94	0.81
IPI00044583	TRERF1	MIDAC	3	1	0	0.92	0.21	0.91	0.11
IPI00057097	DNTTIP1	MIDAC	12	6	0.1	0.92	0.26	0.92	0.11
IPI00103554	GATAD2B	NuRD	14	6	0.16	0.94	0.36	0.92	0.15
IPI00106502	KEAP1		5	2	0.67	0.79	0.82	1.06	0.77
IPI00165357	MTA3	NuRD	3	1	0.08	0.98	0.33	1.04	0.07
IPI00170596	SIN3A	SIN3	23	10	0.09	0.57	0.31	0.83	0.34
IPI00171798	MTA2	NuRD	11	8	0.12	0.63	0.37	0.87	0.14
IPI00215963	GSE1	CoREST	44	13	0.03	0.95	0.22	0.92	0.02

IPI00217540	LSD1	CoREST	79	14	0.05	1.11	0.24	1.06	0.05
IPI00217965	HDAC3		35	10	0.12	0.95	0.28	0.97	0.14
IPI00219695	PTPN2		3	1	0.72	1.03	0.86	1.07	0.64
IPI00239077	HINT1		73	8	0.73	1.62	1.56	1.35	1.31
IPI00289601	HDAC2		90	10	0.11	0.97	0.46	0.97	0.14
IPI00291419	ACAT2		10	4	0.96	0.96	1.02	0.96	0.92
IPI00294603	ZMYM2	CoREST	37	11	0.06	0.91	0.17	0.93	0.06
IPI00301224	TMLHE		2	1	0.22	0.77	0.71	1.02	0.2
IPI00328319	RBBP4	NURD/SIN3	22	5	0.09	0.87	0.28	0.93	0.08
IPI00410351	NCOR1	NCOR	16	7	0.07	0.93	0.13	0.9	0.1
IPI00439194	MBD3	NuRD	6	3	0.12	1.33	0.58	1.12	0.22
IPI00464951	HMG20B	CoREST	27	8	0.03	0.98	0.26	0.98	0.04
IPI00464980	SIN3B	SIN3	6	2	0.07	0.84	0.2	0.92	0.1
IPI00477825	MIER1	SANT	4	1	0.22	0.94	0.37	1.06	0.25
IPI00478128	GATAD2A	NuRD	24	9	0.1	0.96	0.35	0.94	0.14
IPI00640917	TBL1X	NCOR	3	1	0	1.11	0.34	0.92	0
IPI00646512	RBBP7	NURD/SIN3	13	3	0.08	1.1	0.36	1.22	0.13
IPI00657688	MIER3	SANT	10	3	0.14	0.88	0.26	0.92	0.16
IPI00737174	PHF21A	CoREST	5	2	0.18	0.98	0.18	1.06	0.15
IPI00747259	HDAC8		16	4	0.11	0.88	0.29	0.96	0.28
IPI00784154	HSPD1		31	11	0.06	0.81	0.27	0.91	0.06
IPI00784739	MIDEAS	MIDAC	9	4	0.12	0.78	0.18	0.91	0.11
IPI00872209	PPP3CA		8	2	0.72	1.03	0.67	1.08	0.88
IPI00874235	ERGIC1		19	5	0.53	0.91	0.74	0.99	0.46
IPI00914887	RCOR3	CoREST	41	12	0.03	0.95	0.22	0.95	0.02

Supplementary Table 5B: Competition binding experiments of indicated compounds at 10 µM using the N-I-BET matrix

SSM quant: quantified sequence-to-spectrum matches; pep quant: distinct peptides for quantification

					Fold-change relative to vehicle control				
ACCESSION	PROTEIN	ANNOTATION	SSM quant	pep quant	3	I-BET295	DUAL946	19	SAHA
IPI00007334	ACIN1		3	1	0.33	0.13	0	0.07	1.09
IPI00003627	ACTL6A		8	4	0.83	0.73	0.59	0.58	1.11
IPI00004344	AFF4	pTEFb-SEC	2	2	0.41	0.15	0.11	0.09	1.07
IPI00328658	ASH2L		7	5	0.44	0.32	0.26	0.26	1.11
IPI00102575	ATAD5	RFC/RLC	2	1	0.38	0.02	0.13	0.11	1.27
IPI00440502	BRD2	BET	10	5	0.32	0.12	0.1	0.1	1.13
IPI00014266	BRD3	BET	24	10	0.52	0.05	0.04	0.05	1.27
IPI00440727	BRD4	BET	43	13	0.52	0.07	0.05	0.06	1.16
IPI00908444	CAMK2G		15	6	1.05	0.94	0.81	0.83	0.93
IPI00030247	CCNT1	pTEFb-SEC	7	4	0.46	0.13	0.14	0.12	1.07
IPI00300659	CDC73	PAF	14	5	0.34	0.13	0.1	0.1	1.06
IPI00552413	CDK9	pTEFb-SEC	13	5	0.47	0.14	0.14	0.17	1.15
IPI00719073	CHD8	CHROMO	13	7	0.26	0.07	0.06	0.07	0.91
IPI00383105	CHD9	CHROMO	5	4	0.42	0.23	0.17	0.2	0.96
IPI00333010	CHERP		7	5	0.35	0.26	0.19	0.22	1.16
IPI00477468	CTR9	PAF	2	2	0.39	0.3	0.23	0.29	1.06
IPI00020454	DCK		10	5	1.17	1.65	1.15	1.19	1.53
IPI00644431	DDX39		4	2	1.46	0.69	0.61	0.75	1.07
IPI00007208	DDX41		11	6	0.4	0.14	0.13	0.15	1.12
IPI00396435	DHX15		2	2	0.23	0.14	0.14	0.13	0.9
IPI00025753	DSG1		2	1	1.18	0.93	0.95	0.51	0.44
IPI00009328	EIF4A3		5	4	0.3	0.28	0.23	0.22	0.99
IPI00023467	ELL	pTEFb-SEC	7	5	0.36	0.28	0.15	0.17	1.08
IPI00397801	FLG2		2	2	0.72	0.87	1.14	1.05	1.04

IPI00641950	GNB2L1		4	3	0.52	0.46	0.36	0.34	0.99
IPI00292228	GSK3A		35	8	1.02	1.06	1.06	1.05	1.01
IPI00216190	GSK3B		27	7	0.98	0.93	0.94	0.95	0.99
IPI00641743	HCFC1		3	2	0.33	0.28	0.27	0.29	1.11
IPI00012439	HDAC10		3	1	0.3	0.92	0.15	0.66	0.08
IPI00013290	HDGF2	PWWP	4	2	0.25	0.34	0.2	0.23	1.14
IPI00171903	HNRNPM		5	2	0.55	0.57	0.47	0.46	1.11
IPI00003362	HSPA5		7	5	0.67	0.29	0.23	0.36	1.1
IPI00003865	HSPA8		18	7	0.56	0.17	0.14	0.19	1.1
IPI00103090	LEO1	PAF	3	1	0.29	0.05	0.08	0.04	1.16
IPI00100630	MLLT1	pTEFb-SEC	3	2	0.48	0.33	0.18	0.18	1.08
IPI00019380	NCBP1		4	2	0.22	0.17	0.13	0.15	0.99
IPI00216654	NOLC1		12	6	0.26	0.02	0.02	0.04	1.19
IPI00304596	NONO		3	1	0.78	2.81	0.57	1.99	0.42
IPI00300333	PAF1	PAF	6	4	0.35	0.15	0.11	0.16	1.13
IPI00337386	PRPF40A		3	1	0.3	0.17	0.13	0.14	1.08
IPI00007928	PRPF8		7	3	0.5	0.47	0.35	0.29	1.09
IPI00219445	PSME3		7	4	0.49	0.21	0.19	0.22	1.11
IPI00069750	PUF60		5	4	0.42	0.26	0.19	0.16	1.09
IPI00328319	RBBP4		6	3	1	0.99	0.87	0.93	1.27
IPI00478230	RBBP5		6	3	0.4	0.28	0.25	0.31	1.07
IPI00017412	RFC2	RFC/RLC	2	1	0.45	0.2	0.16	0.13	1.04
IPI00021187	RUVBL1		11	4	0.93	0.8	0.81	0.79	1.06
IPI00017451	SF3A1		2	2	0.63	0.59	0.71	0.77	1.15
IPI00017341	SF3A2		5	2	0.45	0.49	0.49	0.4	1.13
IPI00029764	SF3A3		5	3	0.56	0.65	0.6	0.57	1.19
IPI00026089	SF3B1		5	3	0.49	0.42	0.36	0.34	0.95
IPI00300371	SF3B3		3	2	0.52	0.35	0.39	0.34	1.09
IPI00218591	SFRS1		3	1	0.35	0.31	0.38	0.39	1.21
IPI00012345	SFRS6		4	2	0.41	0.3	0.26	0.24	1.1
IPI00868835	SIMILAR TO HETEROGENEOUS		3	1	0.61	0.63	0.67	0.47	1.28

	NUCLEAR RIBONUCLEOPROTEIN								
IPI00647217	SKIV2L2		4	3	0.53	0.43	0.38	0.3	1.08
IPI00234252	SMARCC1	BAF	7	5	0.66	0.68	0.55	0.5	1.16
IPI00420014	SNRNP200		5	2	0.45	0.38	0.36	0.26	1.19
IPI00012382	SNRPA		8	3	0.38	0.24	0.3	0.3	1.1
IPI00879750	SNRPD3		6	2	0.49	0.4	0.33	0.33	1.11
IPI00029266	SNRPE		2	1	0.48	0.54	0.44	0.47	1.04
IPI00782992	SRRM2		10	6	0.39	0.21	0.13	0.14	1.14
IPI00221222	SUB1		5	3	0.36	0.19	0.16	0.25	1.07
IPI00815713	TCOF1		8	4	0.29	0.09	0.07	0.07	1.05
IPI00015924	TFIP11		4	2	0.5	0.59	0.33	0.31	1.17
IPI00104050	THRAP3		2	1	0.28	0.11	0.14	0.08	1.02
IPI00438229	TRIM28		8	5	0.76	0.96	0.85	0.77	1.21
IPI00014533	UBTF		2	1	0.38	0.2	0.18	0.13	1.16
IPI00005492	WDR5		2	2	0.55	0.64	0.37	0.39	0.9
IPI00019269	WDR61	PAF	9	4	0.43	0.23	0.17	0.21	1.02
IPI00152695	WDR82		3	1	0.38	0.15	0.15	0.19	1.08
IPI00845348	ZRANB2		15	4	0.37	0.06	0.05	0.07	1.19

Supplementary Table 6: Dose-dependent competition binding experiments of compounds DUAL946 and 3 using N-I-BET- and N-SAHA matrices

SSM quant: quantified sequence-to-spectrum matches; pep quant: distinct peptides for quantification

EXPERIMENT	COMPOUND	AFFINITY MATRIX	ACCESSION	PROTEIN	ANNOTATION	MASCOT SCORE	SSM quant	pep quant	IC50	pIC50
X017105	DUAL946	N-I-BET	IPI00440727	BRD4	BET	4296	363	75	0.27	6.57
X017143	DUAL946	N-I-BET	IPI00440727	BRD4	BET	4054	319	73	0.23	6.64
X016226	DUAL946	N-SAHA	IPI00013774	HDAC1	HDAC class I	2962	297	38	0.89	6.05
X016226	DUAL946	N-SAHA	IPI00005711	HDAC6	HDAC class IIb	3196	252	47	0.76	6.12
X016226	DUAL946	N-SAHA	IPI00289601	HDAC2	HDAC class I	2956	252	32	1.3	5.89
X017065	DUAL946	N-SAHA	IPI00005711	HDAC6	HDAC class IIb	3135	131	43	0.18	6.74
X017105	DUAL946	N-I-BET	IPI00014266	BRD3	BET	2514	119	37	0.14	6.85
X017065	DUAL946	N-SAHA	IPI00013774	HDAC1	HDAC class I	2236	109	29	1.3	5.89
X017143	DUAL946	N-I-BET	IPI00014266	BRD3	BET	2290	98	36	0.14	6.85
X017065	DUAL946	N-SAHA	IPI00289601	HDAC2	HDAC class I	2219	92	24	1.5	5.82
X016227	DUAL946	N-I-BET	IPI00440727	BRD4	BET	832	82	14	0.24	6.62
X016226	DUAL946	N-SAHA	IPI00013774	HDAC1	HDAC class I	612	71	11	0.33	6.48
X016228	**3**	N-SAHA	IPI00005711	HDAC6	HDAC class IIb	914	66	15	1.3	5.89
X016226	DUAL946	N-SAHA	IPI00289601	HDAC2	HDAC class I	678	65	14	1.6	5.80
X016226	DUAL946	N-SAHA	IPI00217965	HDAC3	HDAC class I	1219	65	18	0.5	6.30
X017105	DUAL946	N-I-BET	IPI00440502	BRD2	BET	2221	64	35	0.097	7.01
X016226	DUAL946	N-SAHA	IPI00005711	HDAC6	HDAC class IIb	848	63	15	0.58	6.24
X016226	DUAL946	N-SAHA	IPI00012439	HDAC10	HDAC class IIb	1123	62	15	0.36	6.44
X016185	**3**	N-I-BET	IPI00440727	BRD4	BET	738	60	13	10	5.00
X017143	DUAL946	N-I-BET	IPI00440502	BRD2	BET	1913	58	33	0.032	7.49
X016228	**3**	N-SAHA	IPI00013774	HDAC1	HDAC class I	634	57	11	0.17	6.77
X016228	**3**	N-SAHA	IPI00012439	HDAC10	HDAC class IIb	783	56	13	14	4.85

X016226	DUAL946	N-SAHA	IPI00217965	HDAC3	HDAC class I	644	53	12	0.67	6.17
X016228	**3**	N-SAHA	IPI00289601	HDAC2	HDAC class I	651	51	11	0.24	6.62
X016226	DUAL946	N-SAHA	IPI00012439	HDAC10	HDAC class IIb	757	48	12	0.33	6.48
X016228	**3**	N-SAHA	IPI00217965	HDAC3	HDAC class I	615	47	13	0.28	6.55
X017065	DUAL946	N-SAHA	IPI00012439	HDAC10	HDAC class IIb	1201	40	17	0.72	6.14
X017065	DUAL946	N-SAHA	IPI00217965	HDAC3	HDAC class I	984	35	17	0.49	6.31
X016185	**3**	N-I-BET	IPI00014266	BRD3	BET	510	30	8	9.4	5.03
X016227	DUAL946	N-I-BET	IPI00014266	BRD3	BET	430	27	7	0.18	6.74
X016227	DUAL946	N-I-BET	IPI00440502	BRD2	BET	372	26	7	0.11	6.96
X016227	DUAL946	N-I-BET	IPI00012439	HDAC10	HDAC class IIb	252	22	6	0.032	7.49
X016228	**3**	N-SAHA	IPI00747259	HDAC8	HDAC class I	501	21	6	0.35	6.46
X016226	DUAL946	N-SAHA	IPI00747259	HDAC8	HDAC class I	532	20	6	n.d.	n.d.
X017065	DUAL946	N-SAHA	IPI00747259	HDAC8	HDAC class I	678	17	9	0.92	6.04
X016185	**3**	N-I-BET	IPI00440502	BRD2	BET	319	14	5	3.7	5.43
X016226	DUAL946	N-SAHA	IPI00747259	HDAC8	HDAC class I	338	13	5	0.21	6.68
X017105	DUAL946	N-I-BET	IPI00012439	HDAC10	HDAC class IIb	575	13	10	0.032	7.49
X017143	DUAL946	N-I-BET	IPI00012439	HDAC10	HDAC class IIb	343	11	8	0.037	7.43
X016226	DUAL946	N-SAHA	IPI00440727	BRD4	BET	530	8	6	n.d.	n.d.
X017065	DUAL946	N-SAHA	IPI00440727	BRD4	BET	642	8	8	n.d.	n.d.
X017105	DUAL946	N-I-BET	IPI00289601	HDAC2	HDAC class I	315	7	4	n.d.	n.d.
X017143	DUAL946	N-I-BET	IPI00289601	HDAC2	HDAC class I	231	7	4	20	4.70
X017105	DUAL946	N-I-BET	IPI00013774	HDAC1	HDAC class I	343	5	5	n.d.	n.d.
X017143	DUAL946	N-I-BET	IPI00013774	HDAC1	HDAC class I	336	5	5	n.d.	n.d.
X016185	**3**	N-I-BET	IPI00012439	HDAC10	HDAC class IIb	140	5	3	0.28	6.55
X017143	DUAL946	N-I-BET	IPI00005711	HDAC6	HDAC class IIb	150	4	4	0.11	6.96
X017105	DUAL946	N-I-BET	IPI00005711	HDAC6	HDAC class IIb	124	3	3	0.1	7.00
X017105	DUAL946	N-I-BET	IPI00217965	HDAC3	HDAC class I	99	3	3	n.d.	n.d.
X016226	DUAL946	N-SAHA	IPI00014266	BRD3	BET	317	2	2	n.d.	n.d.
X017065	DUAL946	N-SAHA	IPI00014266	BRD3	BET	284	2	2	20	4.70
X017065	DUAL946	N-SAHA	IPI00877840	HDAC6		313	2	1	0.15	6.82

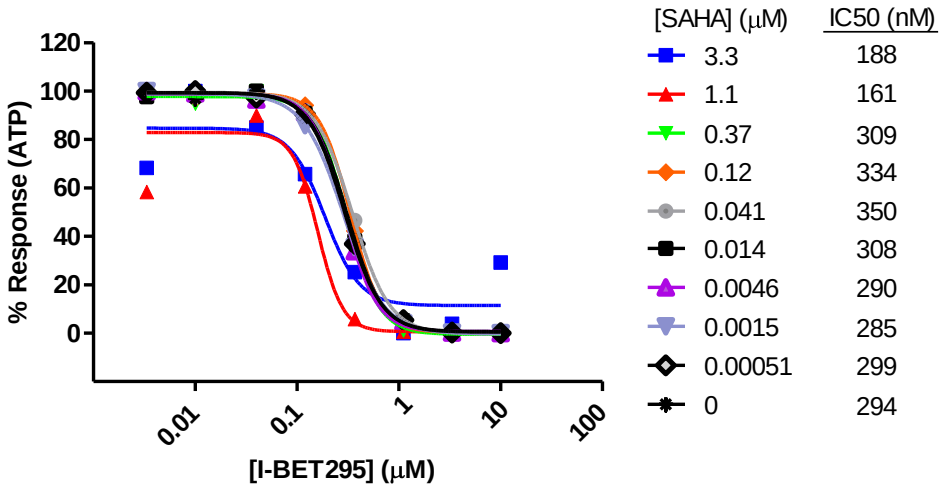
Supplementary Table 7: IC_{50}s and apparent dissociation constants of endogenously expressed BET bromodomain proteins and HDACs.

Chemoproteomic dose-dependent competition binding experiments of compounds DUAL946 and **3** using N-I-BET- and N-SAHA matrices and HL60 cell extracts.

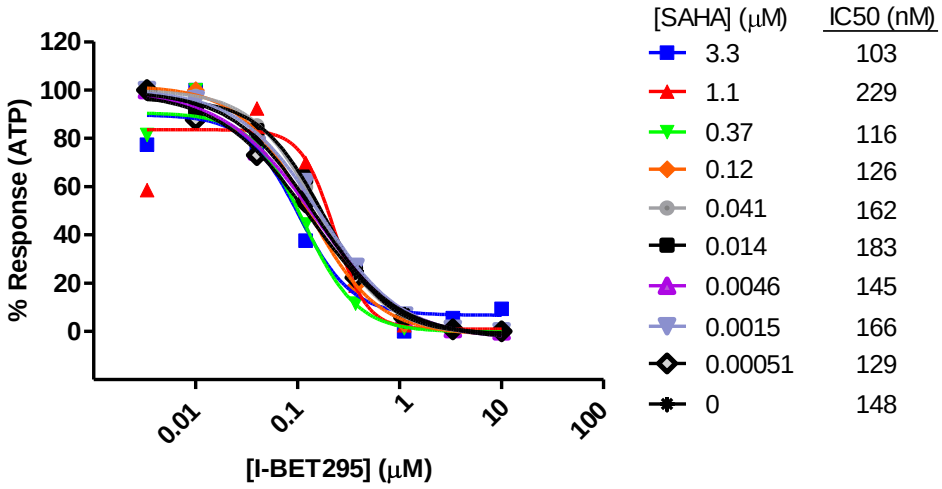
		pIC50				pKdapp				Kdapp (µM)	
	IC50/Kd	**3**		DUAL946		**3**		DUAL946			
protein	correction factor	mean	SEM	mean	SEM	mean	SEM	mean	SEM	**3**	DUAL946
BRD2	2.19[2]	5.43	n.d.	7.16	0.17	5.77	n.d.	7.50	0.18	1.69	0.03
BRD3	3.63[2]	5.03	n.d.	6.82	0.04	5.37	n.d.	7.16	0.04	4.29	0.07
BRD4	2.7[2]	5.00	n.d.	6.61	0.02	5.34	n.d.	6.95	0.02	4.57	0.11
HDAC1	1.68[3]	6.77	n.d.	6.14	0.18	7.11	n.d.	6.48	0.19	0.08	0.33
HDAC2	1.53[3]	6.62	n.d.	5.84	0.03	6.80	n.d.	6.02	0.03	0.16	0.96
HDAC3	1.29[3]	6.55	n.d.	6.26	0.04	6.66	n.d.	6.37	0.04	0.22	0.42
HDAC6	1.82[3]	5.89	n.d.	6.37	0.19	6.15	n.d.	6.63	0.20	0.71	0.24
HDAC8	1.34[3]	6.46	n.d.	6.36	0.32	6.58	n.d.	6.48	0.33	0.26	0.33
HDAC10	2.13[3]	4.85	n.d.	6.36	0.11	5.18	n.d.	6.68	0.11	6.57	0.21

Supplementary Figure 6: Growth inhibition of cancer cell lines. Effect of combinations of SAHA and I-BET295 on the growth of AML cell lines (A) HL60 (n=1) and (B) MV-4-11 (n=1)

A



B



Crystallography Methods:

Crystal structure of Brd4-BD1 with I-BET295 and DUAL946 (1)

E.coli expressed de-His-tagged Brd4-BD1(44-168) was generated as previously described.[4] The protein was at ~10 mg/mL in 10 mM HEPES pH 7.5, 100 mM NaCl and purified to homogeneity using a HisTrap column followed by gel fitration, Tev protease cleavage and gel filtration using a Sephadex 75 column. Compound was added to the protein at 3:1 excess and spun prior to co-crystallisation in 120 nL +120 nL sitting drops @ 20 °C using MRC plates. Crystals were briefly transferred into cryo buffer consisting of the well solution with 20% glycerol or ethylene glycol before flash freezing in liquid nitrogen. For DUAL946, the well solution was 25% PEG6000, 0.1 M trisHCl pH 8.0, 0.2 M LiCl. Data was collected on id23.1 at the ESRF (European Synchrotron Radiation Facility, Grenoble) and processed using XDS and SCALA. For I-BET295, the well solution was 0.1 M bis-tris propane, pH 8.5, 20% PEG3350, 0.2M NaF. Data was collected on an in house FRE+/A200 system and processed using XDS and SCALA. Molecular replacement solution was performed with Phaser (Collaborative Computational Project 4, 1994) using a previous in house apo structure as a starting model of. Model building and refined accomplished using Coot[5] and refmac (Collaborative Computational Project 4, 1994) respectively. The inhibitor compounds could be unambiguously modelled into the excellent difference density shown in supplementary figure 2. Statistics for the data collection and refined co-ordinates are given in Supplementary Table 1. The final models are deposited in the protein data bank under the accession codes 4CLB.pdb (I-BET295/Brd4-BD1) and 4CL9.pdb (DUAL946/Brd4-BD1).

Biochemical Methods

BRD4 Homogenous Time resolved fluorescence assay (HTRF)

Binding was determined by Homogeneous Time Resolved Fluorescence (HTRF) assay as follows: BRD4 protein was diluted to a concentration sufficient to yield a robust signal of at least 3:1 signal:background (~100 nM FAC), into an aliquot of assay buffer (50 mM HEPES, 50 mM NaCl, 0.5 mM CHAPs, pH 7.4, rt). The addition of 2x Kd of an H4 peptide (~300 nM FAC) was made and the mixture was left for 1 h at rt, protected from light. The BRD4-H4 solution was dispensed (8 µL/well, medium speed) using a Multidrop combi, to a black low volume Greiner 384-well plate containing concentration response curves of compound (50 nL/well). This was then left to incubate for 1 h, in the dark at rt. Detection reagents were prepared 15 min prior to use by diluting the Streptavidin-Eu (10 nM FAC) and XL-665 (50 nM FAC) in to detection buffer (50 mM HEPES, 50 mM NaCl, 0.5 mM CHAPs, 150 mM KF, pH 7.4, rt) then added to the assay plate (2 µL/well), followed by a further 1 h incubation. The plates were read on the Envision reader and the donor and acceptor counts were determined. From this, the ratio of acceptor/donor was calculated (λex = 317 nm, λem donor = 615 nm, em acceptor = 665 nm) and used for data analysis. All data was normalized to the robust mean of 16 high (DMSO) and 16 low (inhibitor control: I-BET151) control wells on each plate. A four parameter curve fit of the following form was then applied.

$$y = \frac{a-d}{1+\left(\frac{x}{c}\right)^b} + d$$

Where '*a*' is the minimum, '*b*' is the Hill slope, '*c*' is the pIC50 and'd' is the maximum.

Biophysical Methods

Surface Plasmon Resonance (BIAcore) analysis of DUAL946 binding to BRD4 (1-477) and BRD4-BD2 (338-437)

As described previously,[6] BIAcore data of DUAL946 binding to His6-tagged BRD4 (1-477) and untagged BRD4-BD2 (336-437) was acquired and analysed on a T200 BIAcore instrument at 25 °C. In all cases, a CM5 chip with amine coupled protein was used with typically ~7-10 kRU of protein immobilised on the surface. The running buffer was 10 mM HEPES-NaOH, 150 mM NaCl, 3 mM EDTA, 0.005% Tween 20, pH 7.4 + 1 mM DTT + 1% DMSO. Compounds were titrated as a tripling dilution starting at between 10 μM and 3.3 μM. Sensorgrams and binding curves were analyzed with BIAevaluation (GE Healthcare) using a 1 : 1 binding model. The equilibrium K_D was calculated using a 1 : 1 binding model: Response = Concentation *Rmax/(Concentration+KD) + offset.

Chemoproteomic Methods:

Preparation of cell fractions

Nuclear extract was produced from fresh cells grown in spinner flasks at ~1x10^6 cells/mL. Cells were collected by centrifugation, washed with PBS and resuspended in hypotonic buffer A (10 mM Tris-Cl, pH 7.4, 1.5 mM $MgCl_2$, 10 mM KCl, 25 mM NaF, 1 mM Na_3VO_4, 1 mM DTT, and 1 Roche protease inhibitor tablet per 25 mL). After ca. 3 min the swollen cells were again spun down and resuspended in buffer A and homogenized using a Dounce homogenizer. Nuclei were collected in a microfuge, washed with buffer A and homogenized in one volume of extraction buffer B (50 mM Tris-Cl, pH 7.4, 1.5 mM $MgCl_2$, 20% glycerol, 420 mM NaCl, 25 mM NaF, 1 mM Na_3VO_4, 1 mM DTT, 400 Units/mL DNase I, and 1 Roche protease inhibitor tablet per 25 mL). Extraction was allowed to proceed for 30 min at 4 °C before centrifugation at 13000 *g*. The extract was diluted 3:1 in buffer D (50 mM Tris-Cl, pH 7.4 (RT), 1.5 mM $MgCl_2$, 25 mM NaF, 1 mM Na_3VO_4, 0.6% NP40, 1 mM DTT, and Roche protease inhibitors) and aliquots were stored frozen at -80 °C.

SAHA and I-BET matrix profiling

Experiments are summarized in supplementary tables 5a, 5b & 6. Affinity profiling assays were performed as described previously.[2, 3] Briefly, sepharose beads were derivatized with 1 mM amine-functionalized SAHA[3] or 20 μM N-I-BET[2], washed, and equilibrated in lysis buffer. For each sample 35 μL beads were incubated at 4 °C for 1 h with 1 mL (5 mg) nuclear extract, which had been preincubated with test compound or buffer (vehicle) on an end-over-end shaker. Beads were transferred to disposable columns (MoBiTec), washed with lysis buffer and eluted with SDS sample buffer. Proteins were alkylated with iodoacetamide, separated on 4–12% NuPAGE (Invitrogen), and stained with colloidal Coomassie. Single dose experiments were performed for compounds **3**, **19**, DUAL946, I-BET295, and SAHA using the N-SAHA and N-I-BET matrices (Supplementary Fig. 5). Concentration-dependent competitive binding experiments were performed for DUAL946 (in triplicate) and compound 3 (n=1).

Peptide and protein identification and quantification

Sample preparation, labeling with TMT isobaric mass tags, peptide fractionation, and mass spectrometric analyses were performed essentially as described.[3] Mascot™ 2.0 (Matrix Science) was used for protein identification using 10 ppm mass tolerance for peptide precursors and 20 mDa tolerance for fragment ions. Carbamidomethylation of cysteine residues and TMT modification of lysine residues were set as fixed modifications and methionine oxidation, N-terminal acetylation of proteins and TMT modification of peptide N-termini were set as variable modifications. The search data base consisted of a customized version of the IPI protein sequence database combined with a decoy version of this database created using a script supplied by Matrix Science. For protein quantification a minimum of 2 sequence assignments matching to unique peptides was required. FDR for quantified proteins was <<0.1%. Additional criteria required for peptide quantification were: Mascot ion score > 15, signal to background ratio of the precursor ion > 4, signal to interference > 0.5.[7] Reporter ion intensities were multiplied with the ion accumulation time yielding an area value proportional to the number of reporter ions present in the mass analyzer. Peptide fold changes were corrected for isotope purity as described and adjusted for interference caused by co-eluting nearly isobaric peaks as estimated by the signal-to-interference measure.[7] Protein quantification was achieved using a sum-based bootstrap algorithm.[8] IC50 data were calculated in dose-dependent binding studies using Graphpad Prism and R. Apparent dissociation constants were calculated by eliminating the bead bias from determined IC_{50}s using the Cheng-Prusoff relationship as described.[9]

Cellular Pharmacology Methods:

PBMC Assays:

Peripheral blood mononuclear cells (PBMC) were isolated from healthy volunteer blood. All donors provided written informed consent for use of their samples, and the collection and use of the samples received Institutional Review Board approval.

40000 cells/well (140 µL) were added to 96 well plates containing prediluted test compounds to achieve a range of final assay concentrations (1.52 nM – 10 µM for SAHA and DUAL946, 0.015 nM – 1 µM for I-BET295) in 0.7% DMSO. 0.7% DMSO was also tested as a vehicle control, along with an assay positive standard. Test compounds were incubated for 30 min before the addition of 1 ng/mL LPS (Sigma). Assay plates were then incubated at 37 °C, 5% CO_2 overnight.

100 µL of cell supernatants were removed from the assay plate and analysed for IL-6 and TNFα using MesoScale Discovery (MSD) single plex plates in accordance with the manufacturer's instructions. Plates were read on an MSD Sector Imager 6000 and pg/mL values were backcalculated to standard curves using MSD Discovery Workbench software v4.0.11.

Cell viability was assessed by measuring the ATP content of the remaining cells by the addition of an equal volume of Cell Titre Glo solution (Promega), in accordance with the manufacturer's instructions. The resulting luminescence was measured using a Perkin Elmer Envision 2104 Multilabel reader.

Viability, IL-6 and TNFα data were further normalised to the assay positive and negative controls using Microsoft Excel, and IC_{50} values were generated by nonlinear regression of data from three (DUAL946 and I-BET295) or seven (SAHA) independent experiments using GraphPadPrism (v5).

Cell growth inhibition assays:

11060 and HL60 cells were maintained in RPMI 1640 media (Invitrogen) supplemented with 10% (v/v) FCS (Hyclone) and 5 mM glutamine (Invitrogen). MV-4-11 cells were grown in similarly supplemented IMDM media (Invitrogen).

MV-4-11, HL60 (suspension) or 11060 cells (detached using TrypLE express solution, Invitrogen), were plated in a volume of 90 µL (10,000 cells per well) into 96-well plates (Greiner Microclear) in growth media containing penicillin/streptomycin solution (Invitrogen) and cultured overnight at 37 °C, 5% CO_2. Prior to addition of compounds, one plate of each cell type was removed from the incubator and equilibrated at room temperature for 1 h before assaying for ATP content by the addition of an equal volume of Cell Titre Glo solution (Promega). Luminescence was then measured using a Perkin Elmer Envision 2104 Multilabel reader in order to determine the zero time-point value.

For both single compound additions, and combination experiments, 10 µL of 10x final assay concentrations of test compounds were added to the cell plates. A range of final compound concentrations containing 0.5% DMSO in an assay medium were used. For the single addition experiments (Figure 6) the range of compound concentrations were: 11060 cells - 1.5 nM - 30 µM for SAHA, 7.3 nM – 30 µM for DUAL946 and I-BET295; HL60 and MV-4-11 cells - all compounds 0.51 nM – 10 µM). For combination experiments (Supplementary Fig. 6) in both HL60 and MV-4-11 cells, I-BET295 and SAHA were added to the assay plates in combined concentrations ranges of 0.51 nM - 3.3 µM for SAHA and 0.01 µM – 10 µM I-BET295.

Plates were cultured for a further 72 h at 37 °C, 5% CO_2, and ATP levels were assayed, as described previously. For each cell line, 72 h cell titre glo data were expressed as the percentage of the zero time point value, and then further normalized using GraphPadPrism (v5). IC_{50} values were generated by nonlinear regression of the normalized t=0 data from two (11060 with DUAL946 and I-BET295, HL60 and MV-4-11 with all three compounds) or three (11060 with SAHA) independent experiments using GraphPadPrism (v5).

Western blots:

11060 cells were maintained in RPMI 1640 media (Invitrogen) supplemented with 10% (v/v) FCS (Hyclone) and 5 mM glutamine (Invitrogen). $3x10^6$ cells were plated into 6 cm dishes and cultured overnight. The media was then removed and replaced with fresh media containing either DMSO (0.5%) or IC_{90} concentrations of DUAL946, I-BET295 or SAHA (8.49, 4.60 or 3.65 µM respectively). Duplicate plates were treated with each drug concentration, and were incubated for a further 6 or 24 h. A media change only was performed on one plate of cells for each treatment group, which was processed immediately after addition.

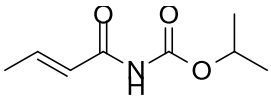
Cells were lysed with RIPA buffer (0.5% (w/v) deoxycholate, 150 mM NaCl, 1% (v/v) NP40, 50 mM TRIS pH 8, 0.1% (w/v) SDS, 10% (v/v) glycerol, 5 mM EDTA) containing Complete Protease inhibitor tablets (Roche), and 15 µg of the lysates were resolved using SDS-PAGE on denaturing Bis tris 4-12% gels (Invitrogen). Gels were transferred to nitrocellulose membranes (Invitrogen) and blocked for 1 h at rt with block buffer (LICOR). Membranes were incubated overnight at 4 °C with primary antibodies (C-myc (Cell Signalling Technology), β-actin (Santa Cruz) and acetyl histone H4 (Millipore)), diluted in block buffer. Membranes were washed and incubated for 1 h at rt with either anti mouse and anti rabbit fluorescently labelled secondary antibodies (Alexa Fluor 680 nm) diluted in wash buffer, and then washed again. Blots were visualised using the LICOR Odyssey Infrared Imaging System.

Chemistry General Procedures

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 were monitored by thin-layer chromatography on 0.2 mm silica gel plates (POLYGRAM SIL G/UV254, Macherey-Nagel) and were visualized with UV light. Compounds were typically purified either by automated flash silica chromatography (Biotage SP4), manual chromatography on pre-packed cartridges (SPE) or by mass directed autopreparative chromatography (MDAP). Where specifically indicated the following formic MDAP method was used: The HPLC analysis was conducted on a Sunfire C18 column (150 mm x 30 mm i.d. 5 µm packing diameter) at ambient temperature, eluting with 0.1% formic acid in water and 0.1% formic acid in acetonitrile using an elution gradient. The UV detection was an averaged signal from wavelength of 210 nm to 350 nm. The mass spectra were recorded on a Waters ZQ Mass Spectrometer using Alternate-scan Positive and Negative Electrospray. Ionisation data was rounded to the nearest integer. ^{1}H and ^{13}C NMR spectra were recorded on either a Bruker DPX 400 MHz or a Bruker AV 600 MHz spectrometer. Chemical shifts are reported in parts per million (ppm, δ units). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad etc. NMR spectra were recorded at ambient temperature (295 K) unless otherwise stated. LC/MS spectra were recorded on an Acquity UPLC BEH C18 column (50 mm x 2.1 mm i.d. 1.7 µm packing diameter) at 40 degrees centigrade. The UV detection was a summed signal from wavelength of 210 nm to 350 nm. The mass spectra were recorded on a Waters ZQ Mass Spectrometer using Alternate-scan Positive and Negative Electrospray. Ionisation data was rounded to the nearest integer. As specifically indicated the compounds were eluted by one of the following LC/MS methods: Formic: eluting with 0.1% v/v solution of Formic Acid in Water (Solvent A) and 0.1% v/v solution of Formic Acid in Acetonitrile (Solvent B) using the following elution gradient 0-1.5 min 3 – 100% B, 1.5-1.9 min 100% B, 1.9 – 2.1 min 3% B at a flow rate of 1 mL/min. High pH: eluting with 10 mM Ammonium Bicarbonate in water adjusted to pH 10 with Ammonia solution (Solvent A) and Acetonitrile (Solvent B) using the following elution gradient 0-1.5 min 1 – 97% B, 1.5-1.9 min 97% B, 1.9 – 2.1 min 100% B at a flow rate of 1 mL/min. TFA: eluting with 0.1% v/v solution of TFA in Water (Solvent A) and 0.1% v/v solution of TFA in Acetonitrile (Solvent B) using the following elution gradient 0-1.5 min 3 – 100% B, 1.5-1.9 min 100% B, 1.9 – 2.0 min 3% B at a flow rate of 1 mL/min. Data are reported with retention time (Rt), m/z of the molecular (MH^+) ion (or a fragment ion if no MH^+ ion was observed) and an estimate of purity, based on the relative areas of peaks in the LC spectrum, as measured by UV detection. High resolution mass spectra were recorded on a

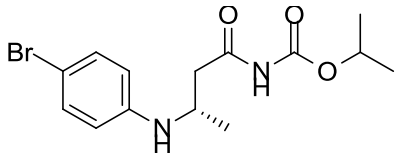
Micromass Q-Tof Ultima hybrid quadrupole time-of-flight mass spectrometer coupled with an Agilent 1100 Liquid Chromatograph. Separations were achieved using a Phenomenex Luna C18(2) reversed phase column (150 x 2.1 mm, 3 µm particle size). Gradient elution was carried out with the mobile phases as (A) water containing 0.1% (v/v) formic acid and (B) acetonitrile containing 0.1% (v/v) formic acid. The flow rate was 0.4 mL/min, temperature controlled at 35 ºC with an injection volume of between 2 to 5 µL. The mass spectrometer was equipped with a Z-spray interface and operated in W reflectron mode. Ionization was achieved with a spray voltage of 3 kV, a cone voltage of 30V, with cone and desolvation gas flows of 5-10 and 500-600 L/hour respectively The elemental composition was calculated using MassLynx v4.0 for the $[M+H]^+$.

(*E*)-Isopropyl but-2-enoylcarbamate (7)



Isopropyl carbamate (30 g, 291 mmol) was charged to a 3L Lara vessel and dry tetrahydrofuran (150 mL) added. (2*E*)-2-Butenoyl chloride (31.2 ml, 326 mmol) was added under nitrogen and the jacket cooled to -30 ºC. When the solution temperature reached -17 ºC, lithium *tert*-butoxide (655 mL, 655 mmol, 1M) was added by peristaltic pump over 2 h, keeping the reaction temperature between -10 ºC and -18 ºC. Once the addition was complete, the mixture was stirred for 30 min and brought to 0 ºC. Diethyl ether (450 mL) and HCl (375 mL, 1M aq.) were added and the mixture brought to 20 ºC with vigorous stirring. The stirring was stopped, the layers allowed to separate and the aqueous layer run off. Brine (375 mL) was added and the mixture stirred vigorously. The stirring was stopped, the layers allowed to separate and the aqueous layer run off. The organic layer was dried ($MgSO_4$), filtered and evaporated to a brown oil (60 g). The mixture was loaded onto a 40+M Biotage silica column and eluted with DCM / ethyl acetate (1:1 -> 0:1, 10CV). The product containing fractions were evaporated to dryness and loaded on to a 1500 g Redisep Isco silica column and eluted with cyclohexane / ethyl acetate (0 -> 40%, 17CV). The clean, product containing fractions were evaporated to an off white solid (15.4 g, 29% yield).
LCMS (TFA) Rt = 0.68 min, MH+ = 172.0 (97% purity)
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.30 (d, *J*=6.3 Hz, 6 H) 1.94 (dd, *J*=6.9, 1.6 Hz, 3 H) 4.94 - 5.06 (m, 1 H) 6.87 (dd, *J*=15.4, 1.5 Hz, 1 H) 7.14 (dq, *J*=15.4, 6.9 Hz, 1 H) 7.35 (br. s., 1 H)

(*S*)-Isopropyl (3-((4-bromophenyl)amino)butanoyl)carbamate (8)



1-Methylethyl (2*E*)-2-butenoylcarbamate (**7**) (9.38 g, 54.8 mmol) was stirred in toluene (281 mL) under nitrogen and ((*R*)-BINAP)ditriflate*bis*(acetonitrile)palladium(II) (3.35 g, 3.01 mmol) added. The catalyst formed a gummy ball, the solution turned to an opaque yellow mixture and was stirred for 20 min. 4-Bromoaniline (14.14 g, 82 mmol) was added, the solution turned a clear light brown and the gummy catalyst dissolved further. The mixture was stirred for 16 h. Similarly a second batch of 1-methylethyl (2*E*)-2-butenoylcarbamate (8.51 g, 49.7 mmol) was stirred in toluene (255 mL) under nitrogen and ((*R*)-

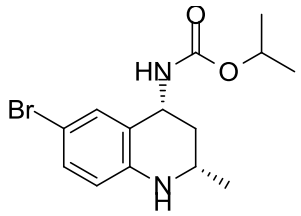
BINAP)ditriflate*bis*(acetonitrile)palladium(II) (3.04 g, 2.73 mmol) added. The catalyst formed a gummy ball, the solution turned to an opaque yellow mixture and was stirred for 20 min. 4-Bromoaniline (12.83 g, 74.6 mmol) was added, the solution turned a clear light brown and the gummy catalyst dissolved further. The mixture was stirred for 16 h. The two reaction mixtures were combined and loaded on to a 1.5 kg Isco silica Rediep column. The column was eluted with DCM / MeOH (0% -> 0.5%, 19CV). The clean, product containing fractions were evaporated to a pale brown oil. The mixture was dried in a vacuum oven overnight at 40 ºC to give a white solid (24.2 g, 67% overall).

LCMS (TFA) Rt = 0.91 min, MH+ = 342.9 (98% purity)

Chiral HPLC: 92%*ee* (Method:~1 mg sample dissolved in EtOH / heptane (1 mL), 20 µL injected on 4.6 mm i.d. x 25 cm Chiralpak AD column (Lot No. AD00CE-FE126). Column eluted with 15% EtOH / heptane, f=1.0 mL/min. UV detector wavelength = 215 nm)

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.26 - 1.35 (m, 9 H) 2.90 (dd, *J*=16.0, 6.2 Hz, 1 H) 3.10 (dd, *J*=15.9, 5.8 Hz, 1 H) 3.86 - 3.94 (m, 1 H) 3.94 - 4.06 (m, 1 H) 4.92 - 5.03 (m, 1 H) 6.51 (d, *J*=8.8 Hz, 2 H) 7.24 (d, *J*=8.8 Hz, 2 H) 7.44 (m, 1 H)

Isopropyl ((2*S*,4*R*)-6-bromo-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate (9)

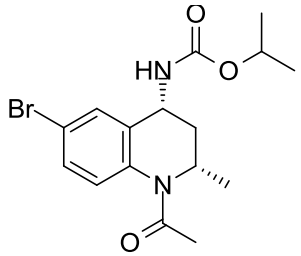


1-Methylethyl {(3*S*)-3-[(4-bromophenyl)amino]butanoyl}carbamate (**8**) (17.9 g, 52.2 mmol) was taken up in ethanol (150 mL) and cooled to below -10 ºC (internal temperature) in a CO_2/acetone bath. Sodium borohydride (1.381 g, 36.5 mmol) was added followed by magnesium chloride hexahydrate (11.35 g, 55.8 mmol) in water (25 mL) keeping the temperature below -5 ºC. The mixture was allowed to stir at < 0 ºC for 1 h then warmed to rt and stirred for 1 h. The resulting thick suspension was poured into a mixture of citric acid (25.05 g, 130 mmol), HCl (205 mL, 205 mmol, 1M aq.) and dichloromethane (205 mL). The biphasic mixture was stirred at rt for 1 h. The layers were separated and the organic layer dried with Na_2SO_4, filtered and concentrated to yield the product as a light brown solid (14.1 g, 78% yield)

LCMS (formic) Rt = 1.13 min, MH+ = 327.2 (99% purity)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.14 (d, *J*=6.3 Hz, 3 H) 1.20 (d, *J*=6.3 Hz, 3 H) 1.23 (d, *J*=6.3 Hz, 3 H) 1.46 (q, *J*=11.9 Hz, 1 H) 1.88 - 1.97 (m, 1 H) 3.40 - 3.51 (m, 1 H) 4.69 - 4.79 (m, 1 H) 4.78 - 4.88 (m, 1 H) 6.48 (d, *J*=8.6 Hz, 1 H) 6.98 (s, 1 H) 7.07 (dd, *J*=8.5, 1.9 Hz, 1 H) 7.42 (d, *J*=9.1 Hz, 1 H)

Isopropyl ((2*S*,4*R*)-1-acetyl-6-bromo-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate (5)



1-Methylethyl [(2*S*,4*R*)-6-bromo-2-methyl-1,2,3,4-tetrahydro-4-quinolinyl]carbamate (**9**) (14.1g, 43.1 mmol) was taken up in dichloromethane (400 mL) under nitrogen at rt. Pyridine (10.46 mL, 129 mmol) followed by acetyl chloride (4.60 mL, 64.6 mmol) were added and the reaction stirred for 16 h. The reaction mixture was partitioned between ethyl acetate (2000 mL) and sat. aq. $NaHCO_3$ (800 mL). The organic layer was extracted and washed with water (1500 mL) and brine (1500 mL) and then dried with Na_2SO_4, filtered and concentrated to yield a purple solid. The crude product was taken up in the minimum of dichloromethane and applied to a Companion XL column (330 g) and eluted with 12% ethyl acetate in cyclohexane for 1CV then 12-63% ethyl acetate over 12CV then held at 63% for 4CV; The appropriate fractions were collected to afford the title product as an off-white solid (12.37 g, 78% yield)
LCMS (formic) Rt = 1.03 min, MH+ = 369.1 (100% purity)
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.01 (d, *J*=6.3 Hz, 3 H) 1.11 - 1.31 (m, 7 H) 2.05 (s, 3 H) 2.44 (ddd, *J*=12.6, 8.6, 4.5 Hz, 1 H) 4.31 - 4.41 (m, 1 H) 4.56 - 4.68 (m, 1 H) 4.83 (spt, *J*=6.2 Hz, 1 H) 7.21 (s, 1 H) 7.30 (d, *J*=8.3 Hz, 1 H) 7.47 (dd, *J*=8.3, 2.0 Hz, 1 H) 7.65 (d, *J*=8.6 Hz, 1 H)

Separation of Enantiomers of 5

Column: Chiralpak AD, 20 micron particle size, internal diameter 75 mm, bed length 258 mm

Eluent: 80:20 heptane : ethanol

Flow: 400 mL/min

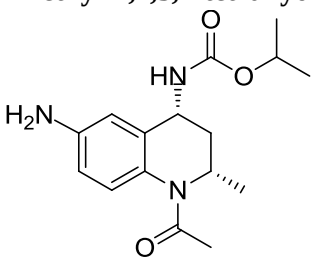
Temperature: Ambient

Injection volume: ~44 mL containing 4 g compound **5** in 35:65 v/v heptane : ethanol.

Run time: 4.5 min.

Pure fractions containing the required first eluting enantiomer from all the injections were concentrated to dryness on a rotary evaporator to give product as a solid. This was slurried in heptane, stirred for 1 h at 20-25 °C, filtered, washed on the filter with heptane and dried under vacuum at 40 °C to give the product as a solid (89% recovery). HPLC analysis showed that the product contained <0.5% of the second eluting enantiomer.

Isopropyl ((2*S*,4*R*)-1-acetyl-6-amino-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate (10)



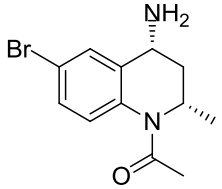
1-Methylethyl [(2*S*,4*R*)-1-acetyl-6-bromo-2-methyl-1,2,3,4-tetrahydro-4-quinolinyl]carbamate (**5**) (3.72 g, 10.07 mmol) and copper (I) oxide (0.392 g, 2.74 mmol) were combined in *N*-methyl-2-pyrrolidone (5 mL) and ammonia solution (5 mL, 264 mmol, 35% in water) was added. The reaction mixture precipitated on addition of the aqueous ammonia. The reaction mixture was then heated at 110 °C in the

microwave (in a 20 mL sealed microwave vial) for 5 h. The reaction mixture was concentrated and partitioned between water and ethyl acetate. The organic layer was separated and the aqueous layer further extracted with ethyl acetate (2 x 50 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to give a brown foamy solid (~3.6 g). The crude product was chromatographed on SiO_2 (Biotage SNAP 100 g cartridge) eluting with 10-100% ethyl acetate/cyclohexane to give 1-methylethyl [(2*S*,4*R*)-1-acetyl-6-amino-2-methyl-1,2,3,4-tetrahydro-4-quinolinyl]carbamate (2.93 g, 8.64 mmol, 86% yield) as an orange foamy solid.

LCMS (formic) Rt = 0.57 min, MH+ = 306.1 (97% purity)

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.95 (d, *J*=6.3 Hz, 3 H) 1.06 - 1.20 (m, 1 H) 1.21 (d, *J*=6.3 Hz, 3 H) 1.24 (d, *J*=6.1 Hz, 3 H) 1.95 (br. s., 3 H) 2.22 - 2.39 (m, 1 H) 4.14 - 4.30 (m, 1 H) 4.52 - 4.73 (m, 1 H) 4.81 (spt, *J*=6.2 Hz, 1 H) 5.13 (s, 2 H) 6.39 (s, 1 H) 6.43 (dd, *J*=8.2, 2.1 Hz, 1 H) 6.87 (d, *J*=8.3 Hz, 1 H) 7.37 (d, *J*=8.8 Hz, 1 H)

1-((2*S*,4*R*)-4-Amino-6-bromo-2-methyl-3,4-dihydroquinolin-1(2*H*)-yl)ethanone (11)

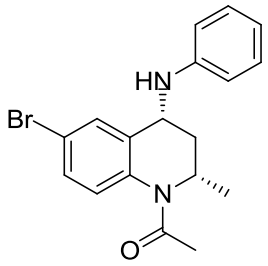


Aluminium chloride (1.23 g, 9.22 mmol) was suspended in dichloromethane (10 mL) and cooled in an ice bath. Isopropyl ((2*S*,4*R*)-1-acetyl-6-bromo-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate (**5**) (1 g, 2.71 mmol) in dichloromethane (6 mL) was added dropwise over ~5 min and the reaction mixture was then left to stir in the ice bath for a further 2.5 h. The reaction mixture was quenched by the addition of water (100 mL) followed by dropwise addition of NaOH (1.14 g, 28.4 mmol) in water (10 mL), whilst keeping the reaction in the ice bath and maintaining an internal temperature <10 °C. Rochelle's salt (3.82 g, 13.54 mmol) was added in water (16 mL) and the reaction mixture stirred overnight. By morning the layers were distinct and separable. The organic layer was separated and the aqueous layer was further extracted with DCM (2 x 50 mL). The combined organic layers were passed through a hydrophobic frit and concentrated to give 1-((2*S*,4*R*)-4-amino-6-bromo-2-methyl-3,4-dihydroquinolin-1(2*H*)-yl)ethanone (752 mg, 2.66 mmol, 98% yield) as a yellow oil.

LCMS (High pH): Rt = 0.78 min, MH+ = 283.1 (97% purity)

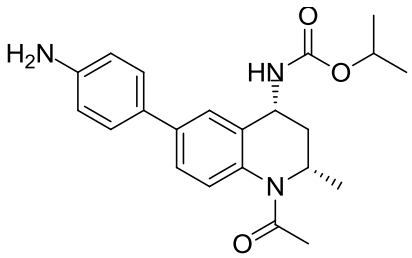
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.04 - 1.16 (m, 1 H) 1.11 (d, *J*=6.3 Hz, 3 H) 2.09 (s, 3 H) 2.53 (ddd, *J*=12.6, 8.5, 4.4 Hz, 1 H) 3.71 (dd, *J*=12.0, 4.4 Hz, 1 H) 4.73 - 4.88 (m, 1 H) 6.98 (d, *J*=8.1 Hz, 1 H) 7.40 (dd, *J*=8.3, 1.5 Hz, 1 H) 7.65 (dd, *J*=2.3, 1.0 Hz, 1 H)

1-((2*S*,4*R*)-6-Bromo-2-methyl-4-(phenylamino)-3,4-dihydroquinolin-1(2*H*)-yl)ethanone (12)



1-((2*S*,4*R*)-4-Amino-6-bromo-2-methyl-3,4-dihydroquinolin-1(2*H*)-yl)ethanone (**11**) (300 mg, 1.059 mmol), iodobenzene (0.289 mL, 2.119 mmol), Davephos (83 mg, 0.212 mmol), and sodium *tert*-butoxide (305 mg, 3.18 mmol) were added to a microwave vial with 1,4-dioxane (12 mL). A steady flow of nitrogen was bubbled through the suspension for 5 min, after which $Pd_2(dba)_3$ (97 mg, 0.106 mmol) was added to the reaction. Nitrogen was bubbled through the suspension for a further 5 min and the mixture was then heated in a microwave at 100 °C for 25 min. During the course of heating the reaction solvent evaporated. The crude solid was therefore dissolved in DCM (100 mL) and water (100 mL) and the layers separated. The aqueous layer was washed with further DCM (2 x 100 mL). The organic extracts were combined, passed through a hydrophobic frit and concentrated *in vacuo* to give the crude product as a dark brown solid. The crude product was loaded in DCM onto a 50 g SNAP silica column and purified by flash chromatography using an SP4 Biotage apparatus, eluting with 20-80% ethyl acetate in cyclohexane. The appropriate fractions were combined and concentrated on a rotary evaporator to give 1-((2*S*,4*R*)-6-bromo-2-methyl-4-(phenylamino)-3,4-dihydroquinolin-1(2*H*)-yl)ethanone (147 mg, 0.409 mmol, 39% yield) as a yellow oil.
LCMS (formic) Rt = 1.17 min, MH+ = 359.1 (92% purity)
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.15 (d, *J*=6.3 Hz, 3 H) 1.22 - 1.33 (m, 1 H) 2.18 (s, 3 H) 2.65 (ddd, *J*=12.4, 8.5, 4.3 Hz, 1 H) 3.75 (d, *J*=7.1 Hz, 1 H) 4.12 - 4.22 (m, 1 H) 4.78 - 4.96 (m, 1 H) 6.63 (d, *J*=7.6 Hz, 2 H) 6.78 (t, *J*=7.5 Hz, 1 H) 7.02 (d, *J*=8.1 Hz, 1 H) 7.21 (dd, *J*=8.6, 7.3 Hz, 2 H) 7.42 (dd, *J*=8.3, 1.8 Hz, 1 H) 7.48 (dd, *J*=2.3, 1.0 Hz, 1 H)

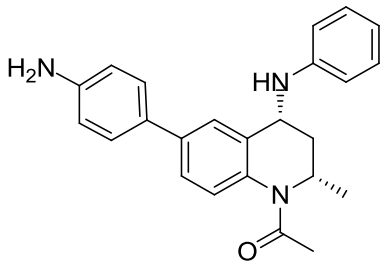
Isopropyl ((2*S*,4*R*)-1-acetyl-6-(4-aminophenyl)-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate (14)



Was prepared from isopropyl ((2*S*,4*R*)-1-acetyl-6-bromo-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate (**5**) (1 g, 2.71 mmol) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**13**) (0.712 g, 3.25 mmol) in a similar manner to **16** to afford the desired product - isopropyl ((2*S*,4*R*)-1-acetyl-6-(4-

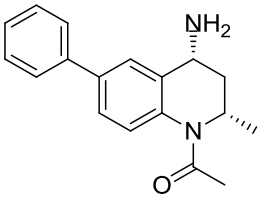
aminophenyl)-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate as a yellow oil (927 mg, 2.430 mmol, 90% yield)
LCMS (formic) Rt = 0.74 min, MH+ = 382.3 (100% purity)
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.17 (d, *J*=6.4 Hz, 3 H) 1.23 - 1.35 (m, 7 H) 2.16 (s, 3 H) 2.61 (ddd, *J*=12.5, 8.4, 4.3 Hz, 1 H) 3.77 (br. s., 2 H) 4.65 - 4.80 (m, 1 H) 4.84 - 4.96 (m, 1 H) 4.96 - 5.07 (m, 1 H) 6.77 (d, *J*=8.6 Hz, 2 H) 7.14 (d, *J*=7.8 Hz, 1 H) 7.35 - 7.49 (m, 4 H)

1-((2*S*,4*R*)-6-(4-Aminophenyl)-2-methyl-4-(phenylamino)-3,4-dihydroquinolin-1(2*H*)-yl)ethanone (15)



Was prepared from 1-((2*S*,4*R*)-6-bromo-2-methyl-4-(phenylamino)-3,4-dihydroquinolin-1(2*H*)-yl)ethanone (**12**) (147 mg, 0.409 mmol) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**13**) (90 mg, 0.409 mmol) in a similar manner to **16** to give 1-((2*S*,4*R*)-6-(4-aminophenyl)-2-methyl-4-(phenylamino)-3,4-dihydroquinolin-1(2*H*)-yl)ethanone (88.3 mg, 0.238 mmol, 58% yield) as a yellow oil.
LCMS (formic) Rt = 0.90 min, (M-NH_3)H+ fragment observed = 279.2 (89% purity)
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.18 (d, *J*=6.4 Hz, 3 H) 1.24 - 1.36 (m, 1 H) 2.22 (s, 3 H) 2.67 (ddd, *J*=12.3, 8.4, 4.4 Hz, 1 H) 3.71 (br. s., 2 H) 3.79 (d, *J*=7.3 Hz, 1 H) 4.21 - 4.30 (m, 1 H) 4.84 - 4.98 (m, 1 H) 6.65 - 6.72 (m, 4 H) 6.76 (t, *J*=7.3 Hz, 1 H) 7.10 - 7.24 (m, 3 H) 7.32 (d, *J*=8.6 Hz, 2 H) 7.44 (dd, *J*=8.2, 1.8 Hz, 1 H) 7.50 (s, 1 H)

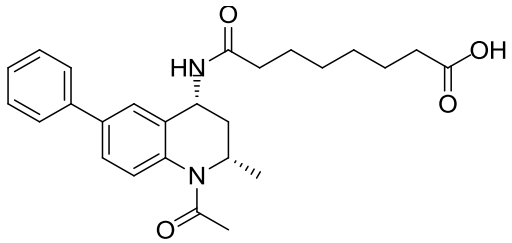
1-((2*S*,4*R*)-4-Amino-2-methyl-6-phenyl-3,4-dihydroquinolin-1(2*H*)-yl)ethanone (16)



1-((2*S*,4*R*)-4-Amino-6-bromo-2-methyl-3,4-dihydroquinolin-1(2*H*)-yl)ethanone (**11**) (200 mg, 0.706 mmol), phenylboronic acid (86 mg, 0.706 mmol) and potassium carbonate (244 mg, 1.766 mmol) were added to a microwave vial with water (1.6 mL) and 1,4-dioxane (9.6 mL). Nitrogen was bubbled through the mixture for 5 min before $PdCl_2$(dppf) (78 mg, 0.106 mmol) was added. Further nitrogen was bubbled through the mixture for 5 min before heating in a microwave at 120 °C for 20 min to give a black suspension. The suspension was separated between DCM (30 mL) and water (30 mL). The aqueous layer was then extracted with further DCM (2 x 30 mL) and the combined organic extracts were dried

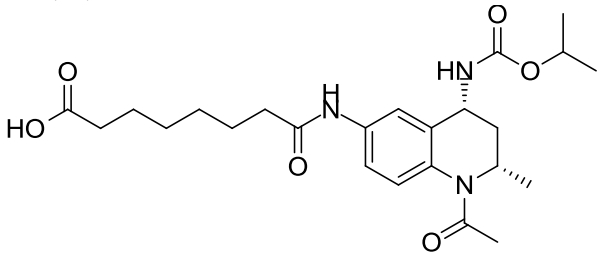
(Na_2SO_4), filtered and concentrated on a rotary evaporator to give a brown oil. This was taken up in MeOH (10 mL) and added to an SCX cartridge (10 g). This was eluted with MeOH (4CV) and the product then eluted with 2M NH_3 in MeOH (4CV). The NH_3/MeOH eluent was concentrated *in vacuo* to afford the product as a brown oil - 1-((2*S*,4*R*)-4-amino-2-methyl-6-phenyl-3,4-dihydroquinolin-1(2*H*)-yl)ethanone (192 mg, 0.685 mmol, 97% yield)
LCMS (High pH): Rt = 0.92 min, (M-NH_3)H+ fragment observed = 264.2 (94% purity)
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.11 - 1.24 (m, 4 H) 2.16 (s, 3 H) 2.57 (ddd, *J*=12.5, 8.5, 4.4 Hz, 1 H) 3.80 (dd, *J*=12.0, 4.4 Hz, 1 H) 4.81 - 4.93 (m, 1 H) 7.18 (d, *J*=8.1 Hz, 1 H) 7.37 (t, *J*=7.3 Hz, 1 H) 7.43 - 7.53 (m, 3 H) 7.64 (d, *J*=7.1 Hz, 2 H) 7.73 (br. s., 1 H)

8-(((2*S*,4*R*)-1-Acetyl-2-methyl-6-phenyl-1,2,3,4-tetrahydroquinolin-4-yl)amino)-8-oxooctanoic acid (17)



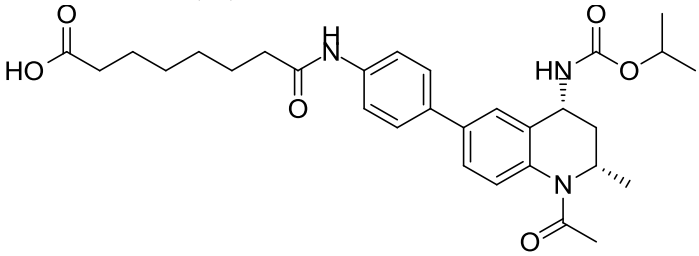
A mixture of CDI (55.5 mg, 0.342 mmol) and DCC (113 mg, 0.548 mmol) in tetrahydrofuran (3 mL) were stirred at rt for 1 h (flask A). In a separate flask was added octanedioic acid (119 mg, 0.685 mmol) and 1-((2*S*,4*R*)-4-amino-2-methyl-6-phenyl-3,4-dihydroquinolin-1(2*H*)-yl)ethanone (**16**) (192 mg, 0.685 mmol) in tetrahydrofuran (1 mL) and the resultant solution added to flask A. The reaction was then left stirring for 64 h. Water (500 µL) was added and the reaction mixture concentrated *in vacuo* to afford the crude product as a beige solid. This was taken up in DMSO/MeOH (1:1, 1.8 mL), divided between 2 vials and purified by formic MDAP. The appropriate fractions were collected and concentrated *in vacuo* to afford the desired product as a white solid - 8-(((2*S*,4*R*)-1-acetyl-2-methyl-6-phenyl-1,2,3,4-tetrahydroquinolin-4-yl)amino)-8-oxooctanoic acid (52 mg, 0.119 mmol, 17% yield)
LCMS (formic): Rt = 0.93 min, MH+ = 437.3 (99% purity)
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.07 (d, *J*=6.4 Hz, 3 H) 1.20 - 1.39 (m, 5 H) 1.49 (quin, *J*=6.7 Hz, 2 H) 1.54 - 1.68 (m, 2 H) 2.10 (s, 3 H) 2.18 (t, *J*=7.3 Hz, 2 H) 2.21 - 2.35 (m, 2 H) 2.45 (ddd, *J*=12.7, 8.4, 4.6 Hz, 1 H) 4.62 - 4.75 (m, 2 H) 7.33 - 7.44 (m, 3 H) 7.48 (t, *J*=7.7 Hz, 2 H) 7.57 (dd, *J*=8.2, 1.8 Hz, 1 H) 7.63 (d, *J*=7.1 Hz, 2 H) 8.26 (d, *J*=8.6 Hz, 1 H) 11.97 (br. s., 1 H)

8-(((2*S*,4*R*)-1-Acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)amino)-8-oxooctanoic acid (18)



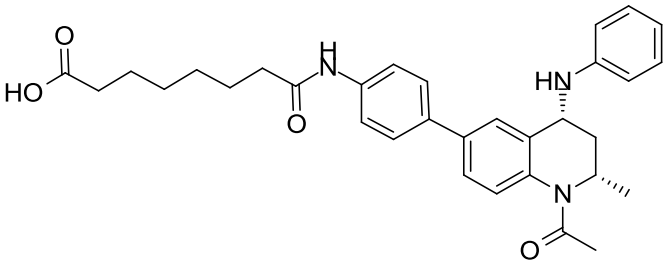
Was prepared from isopropyl ((2*S*,4*R*)-1-acetyl-6-amino-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate (**10**) (500 mg, 1.637 mmol) in a similar manner to **17** to give a clear oil, 8-(((2*S*,4*R*)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)amino)-8-oxooctanoic acid (173 mg, 0.375 mmol, 23% yield).
LCMS (formic): Rt = 0.81 min, MH+ = 462.3 (100% purity)
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.00 (d, *J*=6.3 Hz, 3 H) 1.21 (d, *J*=6.3 Hz, 3 H) 1.25 (d, *J*=6.3 Hz, 3 H) 1.27 - 1.37 (m, 5 H) 1.49 - 1.66 (m, 4 H) 2.00 (s, 3 H) 2.19 (t, *J*=7.3 Hz, 2 H) 2.29 (t, *J*=7.5 Hz, 2 H) 2.38 - 2.46 (m, 1 H) 4.28 - 4.37 (m, 1 H) 4.62 - 4.73 (m, 1 H) 4.84 (spt, *J*=6.2 Hz, 1 H) 6.89 (d, *J*=8.6 Hz, 1 H) 7.12 (d, *J*=8.3 Hz, 1 H) 7.46 (s, 1 H) 7.49 (dd, *J*=8.3, 2.0 Hz, 1 H) 9.49 (br. s., 1 H) 11.47 (br. s., 1 H)

8-((4-((2*S*,4*R*)-1-Acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)phenyl)amino)-8-oxooctanoic acid (19)



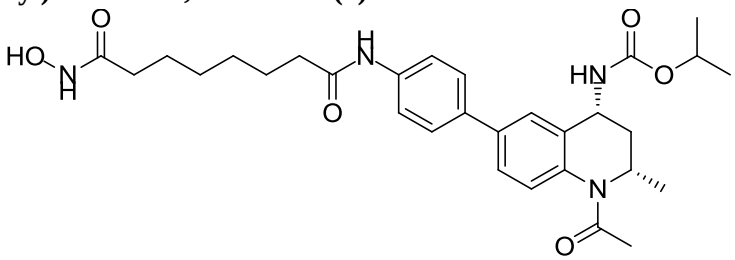
Was prepared from isopropyl ((2*S*,4*R*)-1-acetyl-6-(4-aminophenyl)-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate (**14**) (500 mg, 1.311 mmol) in a similar manner to **17** to afford the desired product as a white solid - 8-((4-((2*S*,4*R*)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)phenyl)amino)-8-oxooctanoic acid (188 mg, 0.350 mmol, 27% yield)
LCMS (formic) Rt = 0.94 min, MH+ = 538.3 (99% purity)
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.04 (d, *J*=6.1 Hz, 3 H) 1.13 - 1.36 (m, 11 H) 1.45 - 1.55 (m, 2 H) 1.55 - 1.64 (m, 2 H) 2.08 (s, 3 H) 2.20 (t, *J*=7.2 Hz, 2 H) 2.32 (t, *J*=7.3 Hz, 2 H) 2.41 - 2.50 (obs, 1 H) 4.35 - 4.46 (m, 1 H) 4.60 - 4.72 (m, 1 H) 4.85 (spt, *J*=6.2 Hz, 1 H) 7.33 - 7.41 (m, 2 H) 7.49 - 7.61 (m, 3 H) 7.63 - 7.74 (m, 3 H) 9.99 (s, 1 H) 12.02 (br. s., 1 H)

8-((4-((2*S*,4*R*)-1-Acetyl-2-methyl-4-(phenylamino)-1,2,3,4-tetrahydroquinolin-6-yl)phenyl)amino)-8-oxooctanoic acid (20)



Was prepared from 1-((2*S*,4*R*)-6-(4-aminophenyl)-2-methyl-4-(phenylamino)-3,4-dihydroquinolin-1(2*H*)-yl)ethanone (**15**) (88 mg, 0.237 mmol) in a similar manner to **17** to give a clear oil, 8-((4-((2*S*,4*R*)-1-acetyl-2-methyl-4-(phenylamino)-1,2,3,4-tetrahydroquinolin-6-yl)phenyl)amino)-8-oxooctanoic acid (59 mg, 0.112 mmol, 47% yield).
LCMS (formic) Rt = 1.03 min, MH+ = 528.3 (100% purity)
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.09 (d, *J*=6.4 Hz, 3 H) 1.17 - 1.35 (m, 5 H) 1.44 - 1.64 (m, 4 H) 2.13 (s, 3 H) 2.19 (t, *J*=7.3 Hz, 2 H) 2.30 (t, *J*=7.5 Hz, 2 H) 2.61 (ddd, *J*=12.3, 8.4, 4.2 Hz, 1 H) 4.22 - 4.32 (m, 1 H) 4.65 - 4.77 (m, 1 H) 6.03 (d, *J*=8.1 Hz, 1 H) 6.58 (t, *J*=7.2 Hz, 1 H) 6.72 (d, *J*=7.8 Hz, 2 H) 7.10 (t, *J*=7.9 Hz, 2 H) 7.37 (d, *J*=8.1 Hz, 1 H) 7.40 - 7.48 (m, 3 H) 7.51 (dd, *J*=8.2, 2.1 Hz, 1 H) 7.63 (d, *J*=8.6 Hz, 2 H) 9.93 (s, 1 H) 11.95 (br. s., 1 H)

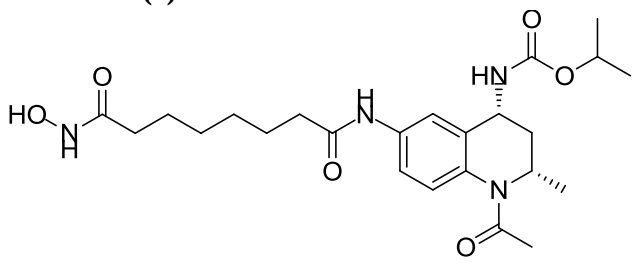
Isopropyl ((2*S*,4*R*)-1-acetyl-6-(4-(8-(hydroxyamino)-8-oxooctanamido)phenyl)-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate, DUAL946 (1)



Was prepared from 8-((4-((2*S*,4*R*)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)phenyl)amino)-8-oxooctanoic acid (**19**) (137 mg, 0.255 mmol) in a similar manner to **3** to afford the desired product as a colourless oil - isopropyl ((2*S*,4*R*)-1-acetyl-6-(4-(8-(hydroxyamino)-8-oxooctanamido)phenyl)-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate (96 mg, 0.174 mmol, 68% yield)
LCMS (formic) Rt = 0.83 min, MH+ = 553.3 (100% purity)
^{1}H NMR (600 MHz, DMSO-*d6*) δ ppm 1.05 (d, *J*=6.2 Hz, 3 H) 1.18 - 1.24 (m, 1 H) 1.21 - 1.34 (m, 4 H) 1.22 - 1.28 (m, 6 H) 1.50 (quin, *J*=7.3 Hz, 2 H) 1.59 (quin, *J*=7.2 Hz, 2 H) 1.95 (t, *J*=7.3 Hz, 2 H) 2.08 (s, 3 H) 2.32 (t, *J*=7.5 Hz, 2 H) 2.43 - 2.49 (m, 1 H) 4.38 - 4.45 (m, 1 H) 4.62 - 4.71 (m, 1 H) 4.86 (spt, *J*=6.2 Hz, 1 H) 7.36 (d, *J*=7.5 Hz, 1 H) 7.37 (s, 1 H) 7.53 (dd, *J*=8.1, 1.8 Hz, 1 H) 7.57 (d, *J*=8.4 Hz, 2 H) 7.63 (d, *J*=8.8 Hz, 1 H) 7.70 (d, *J*=8.4 Hz, 2 H) 8.64 (br. s., 1 H) 9.96 (s, 1 H) 10.32 (br. s., 1 H)
^{13}C NMR (151 MHz, DMSO-*d6*) δ ppm 21.81 (br. s., 1 C) 22.49 (br. s., 1 C) 22.53 (br. s., 1 C) 23.15 (s, 1 C) 25.50 (s, 2 C) 28.89 (s, 2 C) 32.73 (s, 1 C) 36.89 (s, 1 C) 40.03 (s, 1 C) 47.40 (s, 1 C) 47.48 (br. s., 1

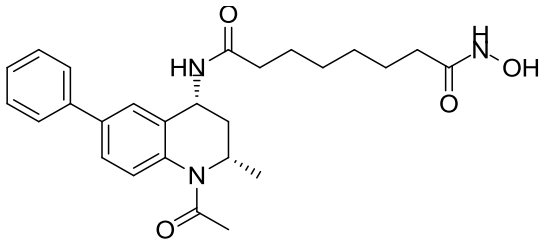
C) 67.67 (s, 1 C) 119.92 (s, 2 C) 120.99 (s, 1 C) 125.04 (s, 1 C) 126.81 (s, 1 C) 127.17 (s, 2 C) 134.78 (s, 1 C) 135.56 (br. s., 1 C) 137.23 (s, 1 C) 137.44 (br. s., 1 C) 139.32 (s, 1 C) 156.37 (s, 1 C) 168.94 (s, 1 C) 169.56 (s, 1 C) 171.79 (s, 1 C)
HRMS $(M+H)^+$ calculated for $C_{30}H_{41}N_4O_6$ 553.3021; found 553.3017.

Isopropyl ((2*S*,4*R*)-1-acetyl-6-(8-(hydroxyamino)-8-oxooctanamido)-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate (2)



Was prepared from 8-(((2*S*,4*R*)-1-acetyl-4-((isopropoxycarbonyl)amino)-2-methyl-1,2,3,4-tetrahydroquinolin-6-yl)amino)-8-oxooctanoic acid (**18**) (100 mg, 0.217 mmol) in a similar manner to **3** to give a yellow solid - isopropyl ((2*S*,4*R*)-1-acetyl-6-(8-(hydroxyamino)-8-oxooctanamido)-2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)carbamate (78.1 mg, 0.164 mmol, 76% yield).
LCMS (formic): Rt = 0.70 min, MH+ = 477.3 (100% purity)
^{1}H NMR (600 MHz, DMSO-*d6*) δ ppm 0.99 (d, *J*=6.2 Hz, 3 H) 1.14 - 1.20 (m, 1 H) 1.22 (d, *J*=6.2 Hz, 3 H) 1.23 - 1.26 (m, 2 H) 1.26 (d, *J*=6.2 Hz, 3 H) 1.28 - 1.32 (m, 2 H) 1.49 (quin, *J*=7.2 Hz, 2 H) 1.53 - 1.61 (m, 2 H) 1.94 (t, *J*=7.3 Hz, 2 H) 2.01 (br. s., 3 H) 2.28 (t, *J*=7.3 Hz, 2 H) 2.35 - 2.42 (m, 1 H) 4.27 - 4.36 (m, 1 H) 4.59 - 4.67 (m, 1 H) 4.83 (spt, *J*=6.2 Hz, 1 H) 7.18 (d, *J*=8.4 Hz, 1 H) 7.45 (s, 1 H) 7.50 (d, *J*=8.8 Hz, 1 H) 7.54 (d, *J*=8.1 Hz, 1 H) 8.62 (br. s., 1 H) 9.93 (s, 1 H) 10.30 (br. s., 1 H)
^{13}C NMR (151 MHz, DMSO-*d6*) δ ppm 21.16 (br. s., 1 C) 22.06 (s, 2 C) 22.46 (s, 1 C) 24.97 (s, 1 C) 25.07 (s, 1 C) 28.35 (s, 2 C) 32.19 (s, 1 C) 36.32 (s, 1 C) 39.82 (br. s, 1 C) 46.46 (br. s., 1 C) 46.85 (s, 1 C) 67.07 (s, 1 C) 113.60 (s, 1 C) 117.11 (br. s., 1 C) 126.00 (br. s., 1 C) 130.72 (br. s., 1 C) 136.88 (br. s., 1 C) 137.32 (br. s., 1 C) 155.69 (s, 1 C) 168.26 (s, 1 C) 169.01 (s, 1 C) 171.08 (s, 1 C)
HRMS $(M+H)^+$ calculated for $C_{24}H_{37}N_4O_6$ 477.2708; found 477.2697.

***N*^{1}-((2*S*,4*R*)-1-Acetyl-2-methyl-6-phenyl-1,2,3,4-tetrahydroquinolin-4-yl)-*N*^{8}-hydroxyoctanediamide (3)**



To a solution of hydroxylamine hydrochloride (23.40 mg, 0.337 mmol) in methanol (0.5 mL) was added potassium hydroxide (28.3 mg, 0.505 mmol) at 0 °C and the reaction stirred for 15 min (flask A). In a separate flask, 8-(((2*S*,4*R*)-1-acetyl-2-methyl-6-phenyl-1,2,3,4-tetrahydroquinolin-4-yl)amino)-8-

oxooctanoic acid (**17**) (42 mg, 0.096 mmol) was dissolved in tetrahydrofuran (1 mL) and cooled to 0 °C. Triethylamine (0.020 mL, 0.144 mmol) and ethyl chloroformate (0.013 mL, 0.135 mmol) were added and the resultant suspension stirred for 15 min at 0 °C (Flask B). The suspension from flask A was added into flask B in one portion and the solution stirred for 30 min at 0 °C and then allowed to warm to rt and stirred for ~3 h. The reaction mixture was allowed to stand over the weekend and then concentrated *in vacuo* and purified by formic MDAP. The appropriate fractions were collected and concentrated *in vacuo* to afford N^1-((2*S*,4*R*)-1-acetyl-2-methyl-6-phenyl-1,2,3,4-tetrahydroquinolin-4-yl)-N^8-hydroxyoctanediamide as a colourless oil (26.8 mg, 0.059 mmol, 62% yield)

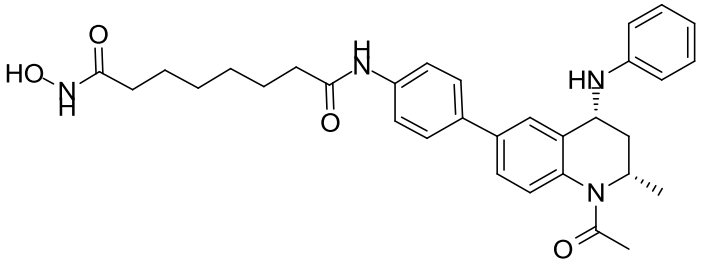
LCMS (formic): Rt = 0.82 min, MH+ = 452.3 (100% purity)

^{1}H NMR (600 MHz, DMSO-*d*6) δ ppm 1.08 (d, *J*=6.2 Hz, 3 H) 1.22 - 1.28 (m, 1 H) 1.25 - 1.36 (m, 4 H) 1.49 (quin, *J*=7.4 Hz, 2 H) 1.54 - 1.68 (m, 2 H) 1.94 (t, *J*=7.5 Hz, 2 H) 2.10 (s, 3 H) 2.19 - 2.27 (m, 1 H) 2.27 - 2.34 (m, 1 H) 2.45 (ddd, *J*=12.7, 8.3, 4.6 Hz, 1 H) 4.63 - 4.76 (m, 2 H) 7.35 - 7.38 (m, 1 H) 7.37 - 7.39 (m, 1 H) 7.41 (d, *J*=8.1 Hz, 1 H) 7.48 (t, *J*=7.7 Hz, 2 H) 7.57 (dd, *J*=8.1, 1.8 Hz, 1 H) 7.63 (d, *J*=7.3 Hz, 2 H) 8.26 (d, *J*=8.4 Hz, 1 H) 8.64 (br. s., 1 H) 10.31 (br. s., 1 H)

^{13}C NMR (151 MHz, DMSO-*d*6) δ ppm 21.81 (s, 1 C) 23.22 (s, 1 C) 25.52 (s, 1 C) 25.95 (s, 1 C) 28.99 (s, 1 C) 29.03 (s, 1 C) 32.77 (s, 1 C) 35.97 (s, 1 C) 40.06 (s, 1 C) 45.19 (s, 1 C) 47.43 (br. s., 1 C) 121.66 (s, 1 C) 125.53 (s, 1 C) 126.94 (s, 2 C) 127.01 (s, 1 C) 127.96 (s, 1 C) 129.52 (s, 2 C) 136.16 (s, 1 C) 137.24 (br. s., 1 C) 137.51 (s, 1 C) 140.25 (s, 1 C) 168.99 (s, 1 C) 169.54 (s, 1 C) 172.74 (s, 1 C)

HRMS $(M+H)^+$ calculated for $C_{26}H_{34}N_3O_4$ 452.2544; found 452.2539.

N^1-(4-((2*S*,4*R*)-1-Acetyl-2-methyl-4-(phenylamino)-1,2,3,4-tetrahydroquinolin-6-yl)phenyl)-N^8-hydroxyoctanediamide (4)



Was prepared from 8-((4-((2*S*,4*R*)-1-acetyl-2-methyl-4-(phenylamino)-1,2,3,4-tetrahydroquinolin-6-yl)phenyl)amino)-8-oxooctanoic acid (**20**) (59 mg, 0.112 mmol) in a similar manner to **3** to give a yellow solid, N^1-(4-((2*S*,4*R*)-1-acetyl-2-methyl-4-(phenylamino)-1,2,3,4-tetrahydroquinolin-6-yl)phenyl)-N^8-hydroxyoctanediamide (5.4 mg, 9.95 µmol, 9% yield).

LCMS (formic) Rt = 0.93 min, MH+ = 543.4 (100% purity)

^{1}H NMR (600 MHz, DMSO-*d*6) δ ppm 1.09 (d, *J*=6.2 Hz, 3 H) 1.19 - 1.24 (m, 1 H) 1.24 - 1.33 (m, 4 H) 1.45 - 1.52 (m, 2 H) 1.53 - 1.61 (m, 2 H) 1.93 (t, *J*=7.3 Hz, 2 H) 2.13 (s, 3 H) 2.29 (t, *J*=7.3 Hz, 2 H) 2.58 - 2.64 (m, 1 H) 4.27 (ddd, *J*=11.8, 8.0, 4.0 Hz, 1 H) 4.63 - 4.80 (m, 1 H) 6.01 (d, *J*=8.1 Hz, 1 H) 6.58 (t, *J*=7.3 Hz, 1 H) 6.72 (d, *J*=8.1 Hz, 2 H) 7.10 (t, *J*=7.9 Hz, 2 H) 7.37 (d, *J*=8.1 Hz, 1 H) 7.43 (s, 1 H) 7.45 (d, *J*=8.4 Hz, 2 H) 7.51 (dd, *J*=8.1, 1.8 Hz, 1 H) 7.63 (d, *J*=8.4 Hz, 2 H) 8.63 (br. s., 1 H) 9.91 (s, 1 H) 10.30 (br. s., 1 H)

^{13}C NMR (151 MHz, DMSO-*d6*) δ ppm 21.26 (br. s., 1 C) 22.83 (s, 1 C) 24.95 (s, 2 C) 28.33 (s, 2 C) 32.18 (s, 1 C) 36.33 (s, 1 C) 40.30 (s, 1 C) 47.09 (br. s., 1 C) 48.70 (s, 1 C) 112.69 (s, 2 C) 116.15 - 116.34 (m, 1 C) 119.22 - 119.44 (m, 2 C) 121.33 - 121.42 (m, 1 C) 124.31 - 124.43 (m, 1 C) 126.31 - 126.40 (m, 1 C) 126.48 - 126.62 (m, 2 C) 128.94 (s, 2 C) 134.31 (s, 1 C) 135.44 (br. s., 1 C) 136.58 (s, 1 C) 138.15 (br. s., 1 C) 138.67 (s, 1 C) 148.20 (s, 1 C) 168.49 (s, 1 C) 169.00 (s, 1 C) 171.19 (s, 1 C)
HRMS $(M+H)^+$ calculated for $C_{32}H_{39}N_4O_4$ 543.2966; found 543.2954.

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