SUPPORTING INFORMATION

Discovery of Small Molecule Inhibitors to Krüppel-Like Factor 10 (KLF10): Implications for Modulation of T Regulatory Cell Differentiation

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Contents:

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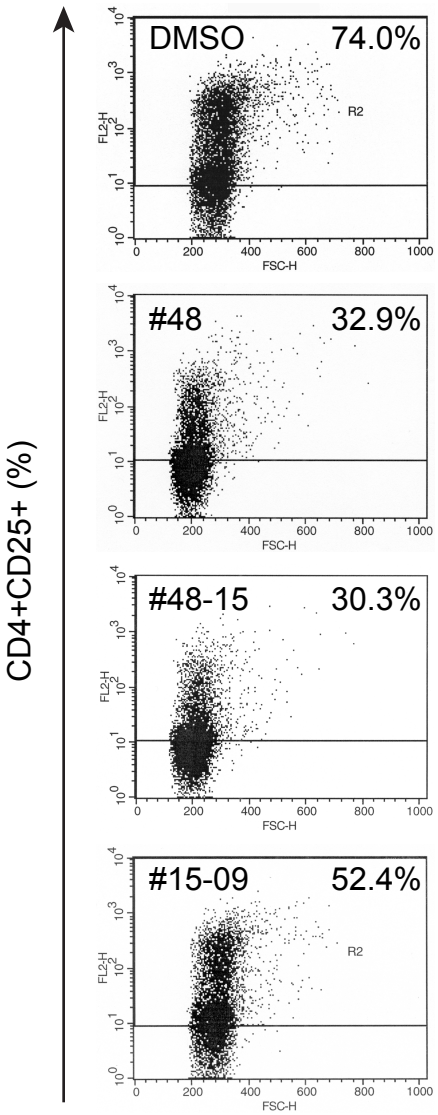
Supplemental Figure 1

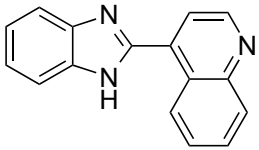
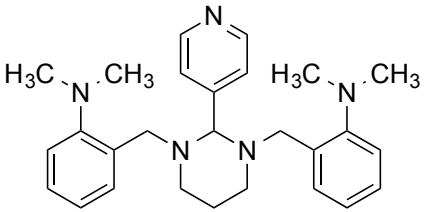
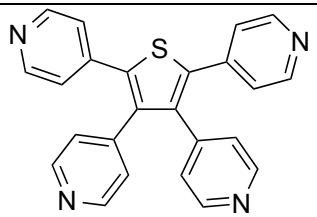


Representative flow cytometry is shown for each condition for Figure 4. Inhibition of conversion of CD4+CD25- T cells to CD4+CD25+ T regs by small molecule compounds #48, #48-15, and #15-09.

Supplemental Figure 2

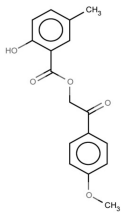
s

Structures of earlier reported inhibitors of other transcription factors as mentioned in the manuscript text.

		
2-(4’-quinoline)-benzimidazole (1)	**GANT61 (2)**	**GANT58 (3)**

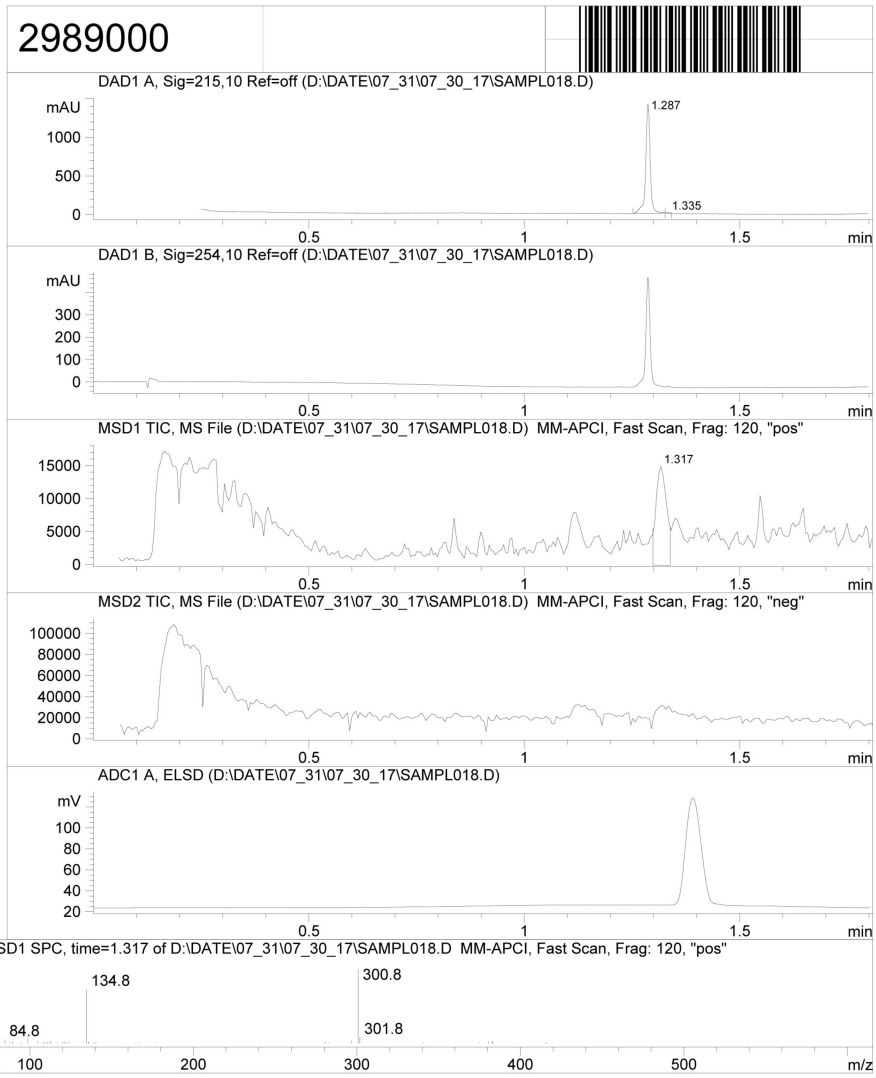
Supplemental Figure 3
#48-15

MaxPeak: 99.38%
Ret_Time: 1.287 min

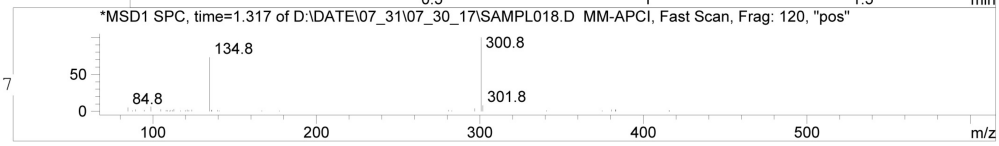


Mol Wt **300.306**
Exact Mass **300.11**

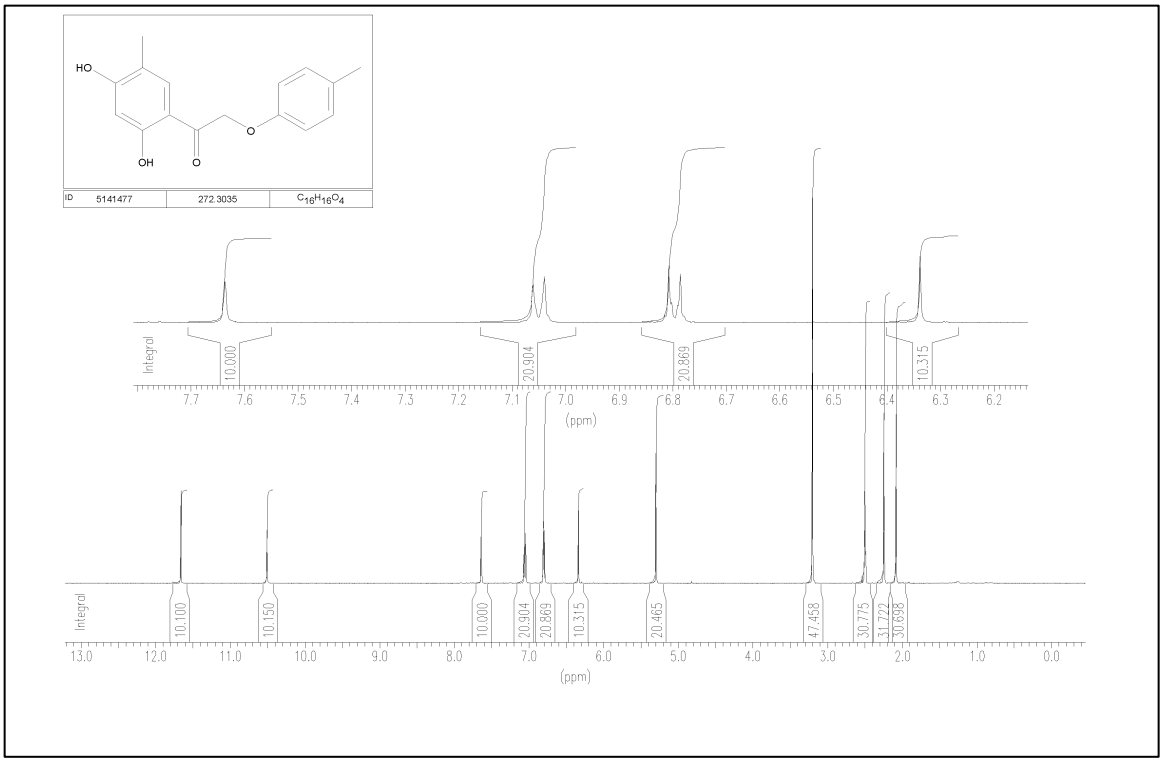
#	Time	Area%
1	1.287	99.38
2	1.335	0.62



RT 1.317

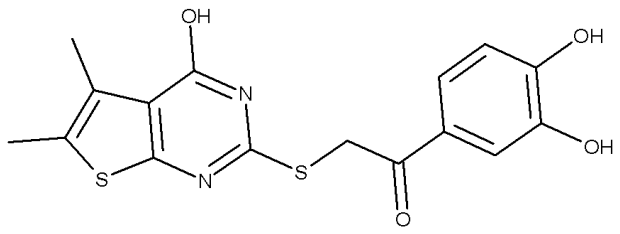


Supplemental Figure 3
#48

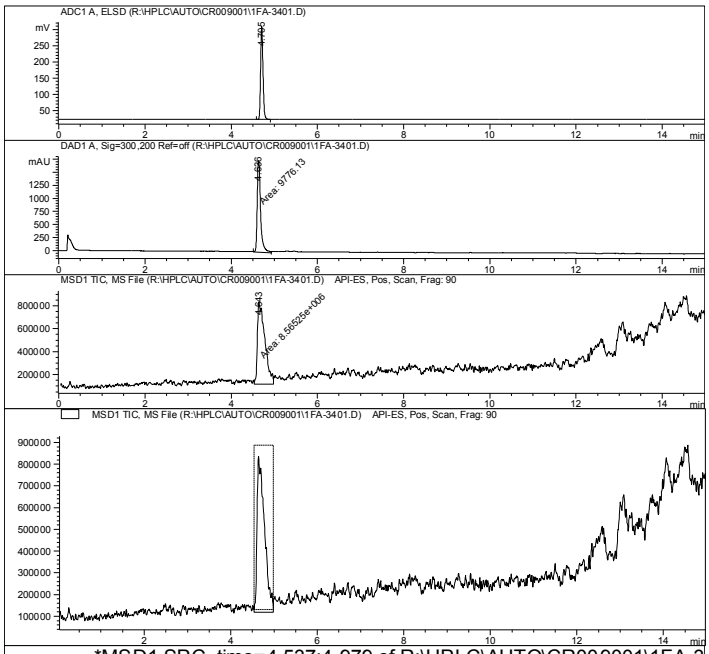


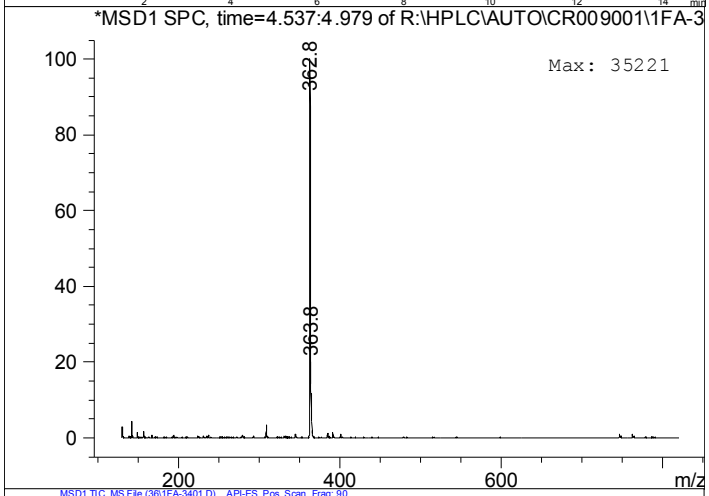
Supplemental Figure 3
#15-09

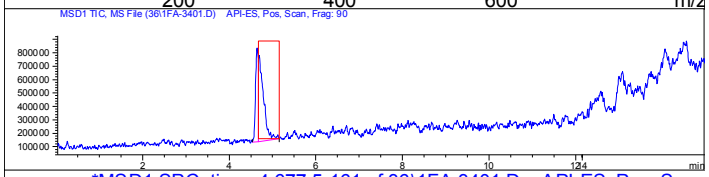
CR00900106

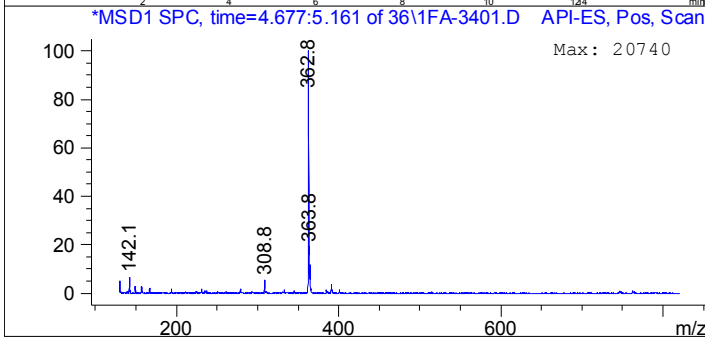


ID	6613772	362.4290	$C_{16}H_{14}N_2O_4S_2$







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Data File R:\HPLC\AUTO\CR009001\1FA-3401.D
Sample Name: CR009001P1-F-01
Instrument 1 25/09/2012 16:04:52
Column: Onyx C18 50x4.6mm | 3.75ml/min | Columns Reg Valve
Gradient: "A"->@14.8min->"B"->@15.min->"A"->PostRun
PMP1, Solvent A          : 0.1%TFA in ACN/H2O (2.5:97.5)
PMP1, Solvent B          : 0.1% TFA in ACN
PMP1, Solvent C          : 0.1%FA in ACN/H2O (2.5:97.5)
PMP1, Solvent D          : 0.1%FA in ACN
Ionization mode              :    API-ES Positive

Signal 1: ADC1 A, ELSD
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mV*s]      [mV]        %
----|-------|----|-------|----------|----------|--------|
   1   4.705 VV    0.0584 1090.21191  286.33060 100.0000
Totals :                  1090.21191  286.33060


Signal 2: DAD1 A, Sig=300,200 Ref=off
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   4.636 MM    0.0934 9776.12793 1744.08984 100.0000
Totals :                  9776.12793 1744.08984


Signal 3: MSD1 TIC, MS File
Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]                          %
----|-------|----|-------|----------|----------|--------|
   1   4.643 MM    0.1986 8.56525e6  7.18809e5  100.0000
Totals :                  8.56525e6  7.18809e5
```

Supplemental Table 1. Raw data for Figure 4 - Inhibition of conversion of CD4+CD25- T cells to CD4+CD25+ T regulatory cells by small molecule compounds #48, #48-15, and #15-09.

	CD4+CD25+ (%)	(%)*
DMSO	70.43	100.00
	75.99	
	75.55	
#48	29.37	44.51
	36.84	
	32.59	
#48-15	39.83	40.92
	16.02	
	34.98	
#15-9	53.92	70.83
	52.70	
	50.61	

*, Values were compared with DMSO condition, which were set to 100 %.

Supplemental Table 2. Raw data for Figure 5 - qPCR Ct values of FoxP3 and β-Actin, and fold changes of FoxP3.

		Average Ct Values		
Cells	**Treatments**	**FoxP3**	**β-Actin**	**Fold change**
CD4+CD25-		28.36	19.77	1
Conversion of CD4+CD25- to CD4+CD25+ cells	DMSO	29.95	23.18	3.52
	#48	33.45	23.97	0.54
	#48-15	32.19	23.02	0.66
	#15-9	36.91	26.82	0.35

SUPPLEMENTAL TABLE 3

Code #	Vendor Code/ Name	Glide-rank-150	GlideXP-Score	Surflex-rank-150	Surflex-Score	FlexX-rank-150	FlexX-Total-Score
AR_001	ZINC00151535	1	-8.28	91	4.35	90	-14.26
AR_002	ZINC00389130	2	-7.57	97	4.27	101	-13.99
AR_003	ZINC00040342	3	-7.30	108	4.15	46	-16.33
AR_004	ZINC00056609	4	-7.25	13	6.10	2	-24.65
AR_005	1-(5-Hydroxy-pyridine-3-c	107	-7.23	30	5.43	140	-12.42
AR_006	ZINC00429664	126	-7.15	107	4.17	41	-16.77
AR_007	796263_1	101	-6.87	33	5.38	39	-16.83
AR_008	ZINC02007905	104	-6.85	128	3.98	20	-18.21
AR_009	ZINC02579359	117	-6.71	25	5.64	8	-21.12
AR_010	4-(3-Hydroxy-propylamin	72	-6.66	144	3.85	31	-17.35
AR_011	ZINC00266187	67	-6.50	50	5.05	112	-13.57
AR_012	ZINC00895472	83	-6.50	42	5.17	129	-12.99
AR_013	ZINC00539105	5	-6.45	92	4.35	25	-17.80
AR_014	ZINC02505891	50	-6.44	63	4.80	123	-13.24
AR_015	7116840_1	34	-6.44	79	4.52	73	-14.95
AR_016	ZINC00895459	57	-6.36	56	4.93	6	-21.86
AR_017	ZINC00062059	98	-6.31	131	3.97	23	-17.97
AR_018	836609_1	40	-6.29	73	4.65	92	-14.26
AR_019	3-(4-Hydroxy-1,1-dioxo-tetra	54	-6.09	59	4.87	103	-13.97
AR_020	ZINC00391975	71	-5.96	48	5.11	29	-17.57
AR_021	ZINC02527749	21	-5.95	11	6.18	131	-12.91
AR_022	ZINC00226748	39	-5.90	74	4.62	5	-22.42
AR_023	7615720_1	121	-5.87	23	5.72	42	-16.74
AR_024	ZINC02040854	12	-5.85	2	6.78	150	-9.61
AR_025	122621	89	-5.84	39	5.25	50	-16.17
AR_026	ZINC00518738	74	-5.80	143	3.86	85	-14.45
AR_027	6910218_1	114	-5.78	123	4.02	15	-19.00
AR_028	ZINC01827766	58	-5.77	55	4.99	143	-11.42
AR_029	ZINC00058791	64	-5.71	148	3.82	34	-17.17
AR_030	ZINC00902215	79	-5.70	44	5.16	99	-14.09
AR_031	ZINC01827765	29	-5.69	84	4.46	142	-12.15
AR_032	134478	17	-5.69	7	6.59	126	-13.10
AR_033	ZINC02566273	125	-5.69	21	5.74	82	-14.62
AR_034	5790116_1	70	-5.63	145	3.85	81	-14.66
AR_035	ZINC00402807	91	-5.61	38	5.27	60	-15.87
AR_036	ZINC02522644	51	-5.60	62	4.81	115	-13.52

AR_037	5131602_1	63	-5.59	52	5.04	40	-16.81
AR_038	ZINC02450093	25	-5.59	88	4.39	109	-13.68
AR_039	ZINC00895334	99	-5.59	34	5.37	7	-21.71
AR_040	ZINC00152602	38	-5.57	75	4.61	53	-16.06
AR_041	ZINC00401167	47	-5.55	66	4.79	119	-13.35
AR_042	ZINC02040855	143	-5.52	15	5.94	97	-14.10
AR_043	ZINC00317204	68	-5.46	146	3.83	136	-12.72
AR_044	ZINC02575475	137	-5.45	17	5.90	27	-17.68
AR_045	ZINC00392006	19	-5.40	9	6.34	89	-14.30
AR_046	1500137	53	-5.39	60	4.85	21	-18.13
AR_047	41355	26	-5.38	87	4.41	66	-15.53
AR_048	5141477	59	-5.37	54	5.00	121	-13.30
AR_049	ZINC00143501	110	-5.30	125	4.00	80	-14.68
AR_050	ZINC02545129	85	-5.29	41	5.19	45	-16.39
AR_051	ZINC00524386	144	-5.26	96	4.28	18	-18.38
AR_052	ZINC02458678	136	-5.19	114	4.09	138	-12.44
AR_053	ZINC02105896	11	-5.15	1	7.96	63	-15.77
AR_054	5483658	139	-5.09	113	4.10	128	-13.02
AR_055	ZINC00968043	113	-5.09	27	5.54	58	-15.88
AR_056	ZINC00140411	94	-5.06	133	3.96	13	-19.23
AR_057	ZINC00366295	6	-5.05	94	4.31	124	-13.17
AR_058	ZINC00093281	75	-5.03	46	5.12	9	-20.75
AR_059	ZINC00075602	149	-5.02	100	4.24	38	-16.98
AR_060	350191	97	-5.02	35	5.35	79	-14.70
AR_061	ZINC00402806	37	-5.01	76	4.60	37	-17.09
AR_062	6366304	32	-4.99	81	4.51	4	-23.57
AR_063	ZINC00119457	14	-4.95	4	6.72	10	-20.38
AR_064	6091005	61	-4.90	53	5.04	36	-17.14
AR_065	5266104	82	-4.88	139	3.89	106	-13.85
AR_066	ZINC00354972	138	-4.88	103	4.19	43	-16.68
AR_067	(4-Hydroxy-1,1-dioxo-tetrah	52	-4.88	61	4.83	146	-11.20
AR_068	ZINC00970164	86	-4.74	137	3.91	74	-14.94
AR_069	ZINC02166236	49	-4.73	64	4.80	137	-12.50
AR_070	163326	87	-4.73	40	5.21	44	-16.46
AR_071	6318933	84	-4.71	138	3.91	116	-13.46
AR_072	ZINC00161424	92	-4.70	134	3.94	12	-19.94
AR_073	ZINC02015035	119	-4.70	24	5.68	59	-15.88
AR_074	5258649	27	-4.69	86	4.42	84	-14.46

AR_075	ZINC00188127	95	-4.69	36	5.35	26	-17.77
AR_076	5-Guanidino-2-[(5-oxo-py	13	-4.67	3	6.73	3	-24.43
AR_077	5738812	135	-4.67	104	4.19	33	-17.28
AR_078	ZINC00161426	127	-4.65	117	4.07	49	-16.22
AR_079	ZINC00170344	122	-4.65	119	4.05	95	-14.15
AR_080	16535	131	-4.64	19	5.81	71	-15.07
AR_081	ZINC02140959	93	-4.64	37	5.31	77	-14.81
AR_082	5260802	78	-4.62	141	3.88	30	-17.39
AR_083	ZINC00582079	118	-4.62	121	4.03	78	-14.72
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AR_085	5233960	81	-4.57	43	5.17	76	-14.84
AR_086	5760308	133	-4.53	115	4.09	35	-17.16
AR_087	ZINC02111574	140	-4.53	16	5.91	75	-14.92
AR_088	ZINC02385763	16	-4.53	6	6.68	17	-18.92
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AR_092	1503938	115	-4.49	26	5.60	127	-13.02
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AR_094	1502155	22	-4.48	12	6.18	1	-28.37
AR_095	ZINC00156508	90	-4.46	135	3.92	22	-18.10
AR_096	5626437	8	-4.41	109	4.15	65	-15.66
AR_097	ZINC00039035	20	-4.39	10	6.22	64	-15.66
AR_098	ZINC00901817	132	-4.38	105	4.19	105	-13.89
AR_099	14161	128	-4.37	20	5.78	61	-15.82
AR_100	ZINC00193719	124	-4.37	118	4.07	48	-16.24
AR_101	ZINC00967235	88	-4.35	136	3.92	144	-11.36
AR_102	ZINC02216764	73	-4.35	47	5.12	19	-18.26
AR_103	ZINC00035764	116	-4.33	122	4.03	87	-14.38
AR_104	ZINC00612242	96	-4.31	132	3.97	57	-15.89
AR_105	ZINC00967233	108	-4.30	126	4.00	52	-16.13
AR_106	ZINC00154988	62	-4.26	149	3.81	110	-13.67
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AR_108	186915	134	-4.21	18	5.84	51	-16.14
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AR_111	ZINC00000558	31	-4.16	82	4.51	102	-13.97
AR_112	ZINC00401915	30	-4.16	83	4.47	56	-15.92

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AR_115	ZINC00967837	44	-4.13	69	4.74	120	-13.33
AR_116	ZINC00043862	45	-4.12	68	4.77	135	-12.72
AR_117	ZINC00900185	80	-4.12	140	3.89	100	-14.02
AR_118	ZINC00050134	9	-4.10	93	4.34	93	-14.22
AR_119	ZINC00001762	103	-4.08	32	5.39	130	-12.98
AR_120	ZINC00035767	130	-4.06	116	4.09	147	-10.56
AR_121	5155819	150	-4.05	110	4.12	96	-14.15
AR_122	ZINC00895060	111	-4.04	28	5.50	111	-13.67
AR_123	ZINC00268033	42	-4.03	71	4.71	69	-15.29
AR_124	ZINC00092200	41	-4.02	72	4.67	118	-13.35
AR_125	ZINC00376788	102	-4.00	129	3.98	55	-15.93
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AR_127	ZINC01584114	15	-3.98	5	6.71	62	-15.82
AR_128	ZINC00899004	76	-3.97	142	3.87	134	-12.75
AR_129	136035	18	-3.97	8	6.36	72	-15.07
AR_130	ZINC00156821	100	-3.96	130	3.98	67	-15.49
AR_131	ZINC00192456	55	-3.94	58	4.88	113	-13.57
AR_132	ZINC00226260	43	-3.94	70	4.74	11	-20.13
AR_133	ZINC01669687	7	-3.94	99	4.25	122	-13.27
AR_134	ZINC02584441	105	-3.94	31	5.41	70	-15.10
AR_135	ZINC00388969	23	-3.94	90	4.36	149	-10.01
AR_136	ZINC00169710	106	-3.93	127	3.99	47	-16.28
AR_137	4-(3-Methoxy-propylamino)-	109	-3.92	29	5.46	132	-12.79
AR_138	ZINC00051582	24	-3.91	89	4.39	145	-11.33
AR_139	ZINC00392420	69	-3.91	49	5.08	54	-16.06
AR_140	ZINC00488339	146	-3.91	111	4.12	133	-12.75
AR_141	664900	35	-3.90	78	4.55	108	-13.80
AR_142	ZINC00407921	123	-3.89	22	5.74	98	-14.10
AR_143	ZINC00156841	65	-3.88	51	5.05	107	-13.84
AR_144	ZINC00358668	141	-3.81	102	4.23	32	-17.30
AR_145	ZINC02257536	28	-3.81	85	4.46	94	-14.18
AR_146	ZINC00154204	129	-3.81	106	4.18	68	-15.40
AR_147	ZINC00006220	60	-3.80	150	3.35	104	-13.93
AR_148	ZINC00156565	142	-3.80	112	4.11	16	-18.96
AR_149	ZINC00057542	48	-3.76	65	4.80	125	-13.13
AR_150	ZINC00322432	36	-3.67	77	4.59	88	-14.36