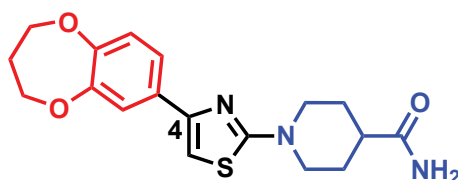


CID-990823
Hit

Exploration of phenyl ring SAR:

Electron-donating groups of 3 or 4 positions increase potency



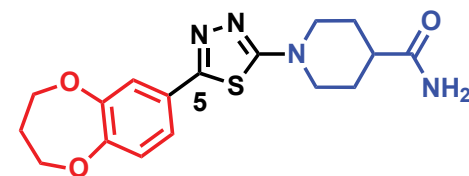
CID-46907714

Backup series:

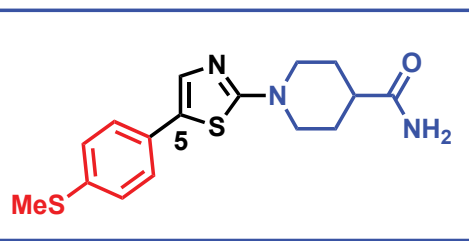
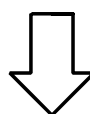
Thiadiazole or pyrimidine

Exploration of thiazole ring SAR:

2,5 substitution of the thiazole dramatically increase potency



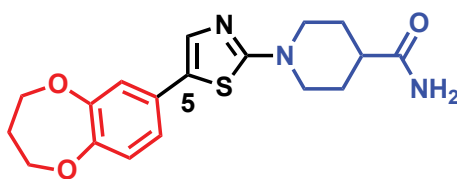
CID-46907746
Backup series



ML200

Fine tune of phenyl SAR:

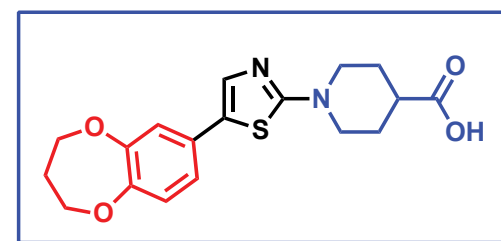
Phenyl substitutions increase efficacy and reduce luciferase activity



CID-46907699

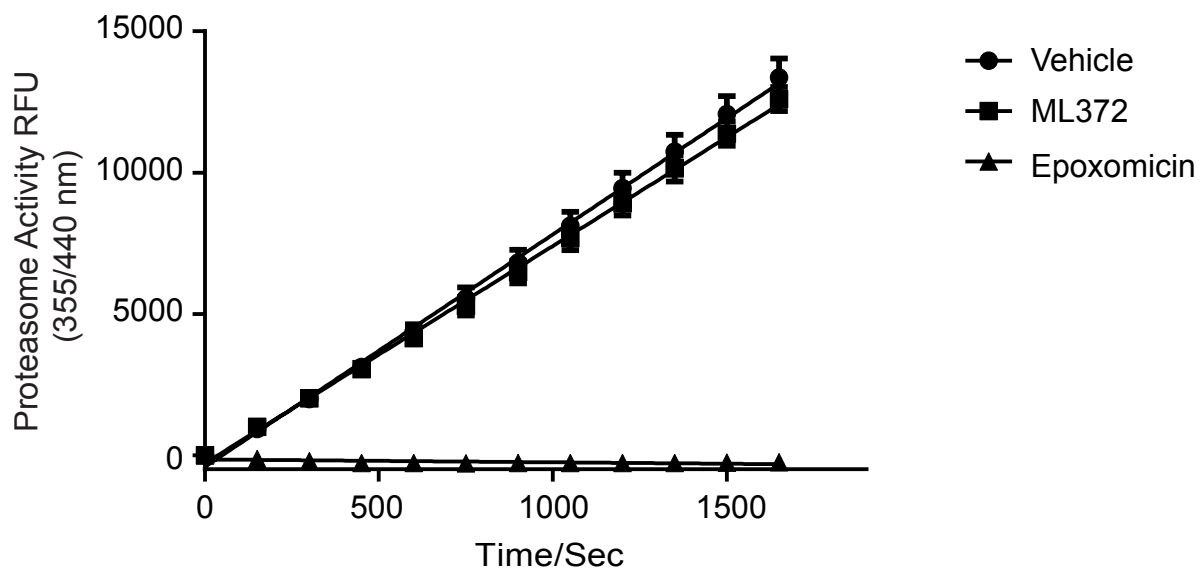
Exploration of piperidine ring SAR

Piperidine ring and carbonyl group are important for SMN2 activity

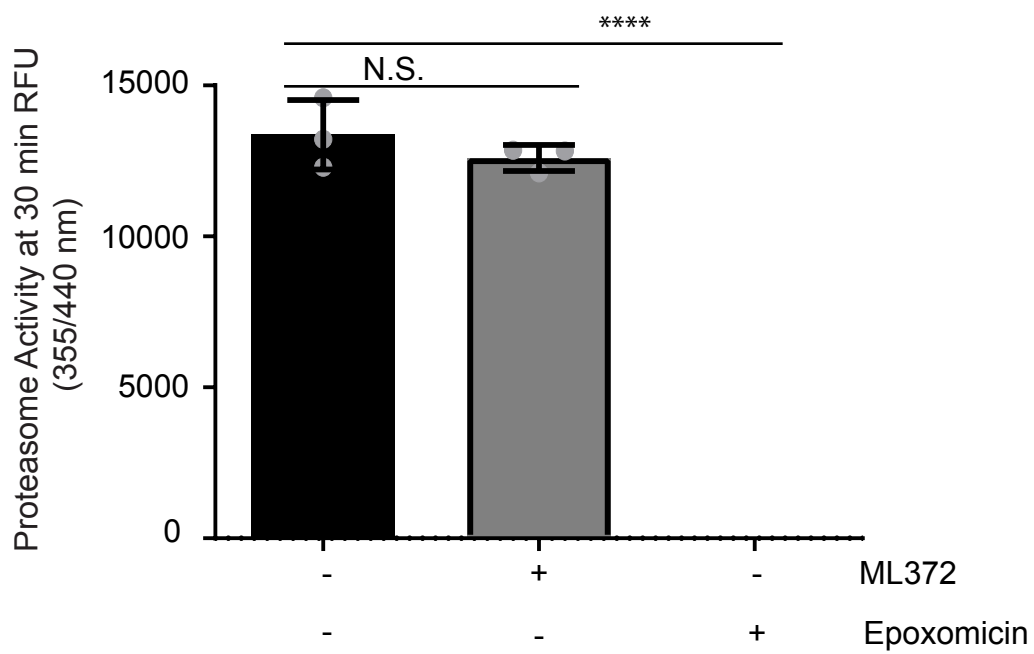


Lead
ML372

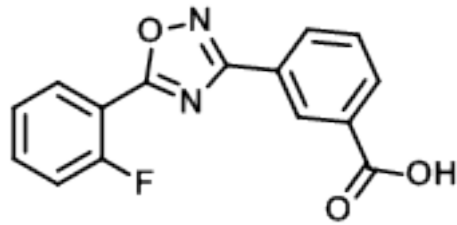
A



B

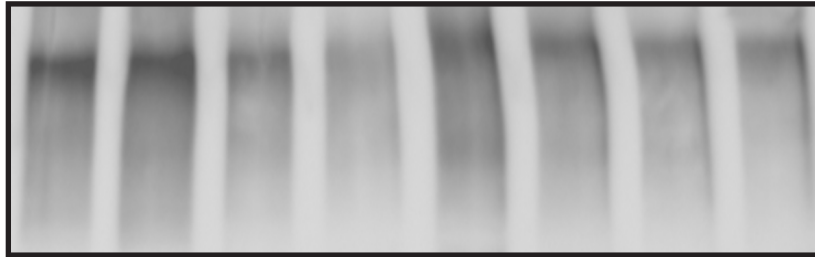


A

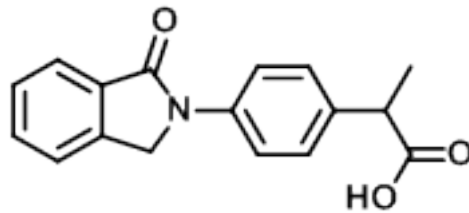


NCGC00168759-05/
PTC124

Mib1	+	+	+	+	+	+	+	+
SMN	+	+	+	+	+	+	+	+
NCGC00168759-05/ PTC124	-	0.1	0.3	1	3	10	30	100

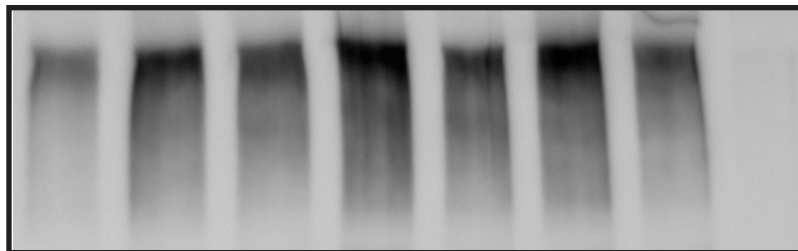


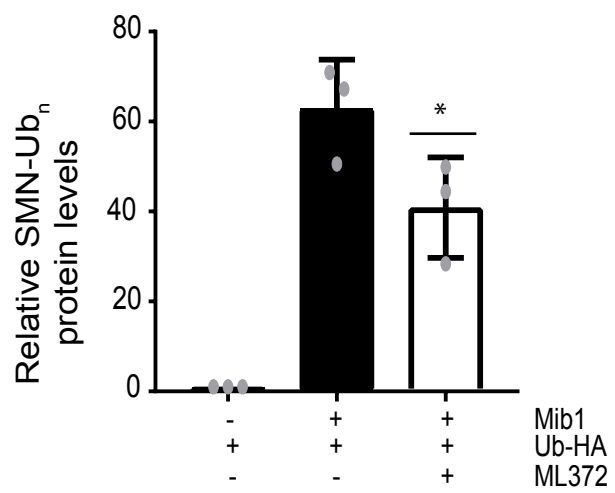
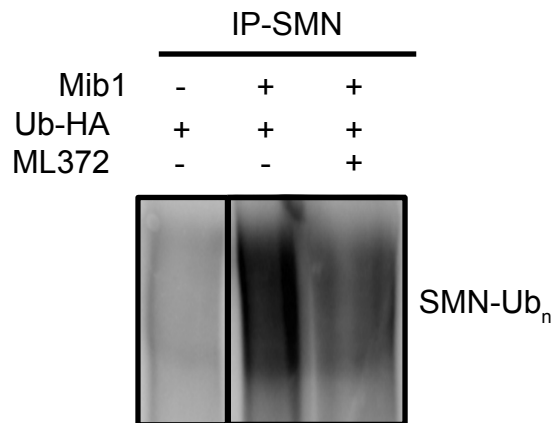
B



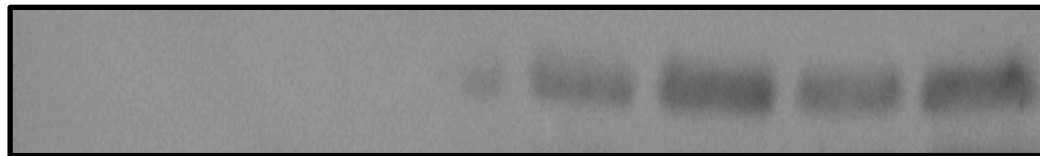
NCGC00094700-07/
Indoprofen

Mib1	+	+	+	+	+	+	+	+
SMN	+	+	+	+	+	+	+	+
NCGC00094700-07/ Indoprofen	-	0.1	0.3	1	3	10	30	100





E1	+	+	-	+	+	+	+	+
Ubch5b	+	+	+	-	+	+	+	+
ATP	-	+	+	+	+	+	+	+
Ubiquitin	+	-	+	+	+	+	+	+
ML372	-	-	-	-	-	0.3	1	3



Ubiquitin-E2 conjugate

Supplemental Table 1. ML372 is found in brain tissues of SMN Δ 7 SMA mice after administration of 50 mg/kg.

Animal ID	Genotype of mice	ML372 level in brain tissue (ng/g)	ML372 level in brain tissue (mmol/kg)
1	SMA	10490	29.14
2	SMA	13508	37.52
3	SMA	3475	9.65
4	SMA	13185	36.63
5	SMA	5439	15.11
6	SMA	8757	24.33
7	Unaffected	10992	30.53
8	Unaffected	13878	38.55

Supplemental Table 2. ML372 binding kinetics with the ubiquitin activating enzyme E1, ubiquitin conjugating enzyme UBCH5b and the E3 ligase Mib1.

	UBA1	UBCH5b	Mib1
Analyte injection concentrations (μ M)	0.352, 1.06, and 3.19	0.122, 0.242, and 0.485	0.156, 0.311, and 0.622
Association rate constant (K_a , 1/Ms)	No binding	No binding	1.77e5
Dissociation rate constant (K_d , 1/s)	No binding	No binding	0.0278
KD (M)	No binding	No binding	1.57e-7