Small molecule inhibition of the muscarinic M1 acetylcholine receptor by potent, selective antagonists facilitate OPC differentiation

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*Michael M Poon[1], Kym I Lorrain[1], Ariana O Lorenzana[1], Karin J Stebbins[1], Alexander Broadhead[1], Thomas O Schrader[2], Yifeng Xiong[2], Jill Baccei[2], Ari J Green[4], Jonah R Chan[4], Daniel S Lorrain[3]; [1]Biol., [2]Chem., [3]Pipeline Therapeutics, San Diego, CA; [4]Neurol., UCSF, San Francisco, CA

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Introduction

Inhibition of the muscarinic acetylcholinergic receptors by non-selective muscarinic antagonists (e.g., clemastine, benztropine) accelerates the differentiation of oligodendrocyte precursor cells (OPCs) into oligodendrocytes (OLs). Subsequent work has implicated the M1 isoform as being a key driver of this phenomenon. In-house chemistry efforts have identified a number of potent, selective M1 antagonists. Using these, we have characterized the effects of inhibiting M1 in a diverse set of *in vitro* assays, including OPC differentiation, cortical myelination, and organotypic brain slice. Our data show that a selective, small molecule inhibitor of M1 is sufficient to drive OPCs towards differentiation and that the resulting oligodendrocytes express myelin basic protein. Moreover, these OLs are functional, i.e., capable of axonal wrapping and induction of nodes of Ranvier. Of note, an M3 selective antagonist (Sagara et al., 2006) was not active in a rat OL differentiation assay. In concert with our *in vivo* data (also presented at this meeting), a strong case can be made that the development of an M1 selective small molecule antagonist is a promising approach for treating demyelinating diseases such as multiple sclerosis.

[^{3}H]NMS membrane binding

Compound	M1 Avg Ki (nM)	Fold selectivity against M1			
		M2/M1	M3/M1	M4/M1	M5/M1
Benztropine	1.14	16	2.67	8.21	2.7
PIPE-359	0.144	130	14.4	45.1	17.4
PIPE-307	0.349	73	18.5	38	259
Compound 57	1.13	22	7.11	29.9	5.37
Compound 25	1.41	160	8.81	189	736
Compound 77	1.48	8.8	41.1	13.5	54.5
Compound 51	2.34	390	113	148	538
Compound 29	2.55	90	17.4	1.95	6.07
Compound 14	3.6	>7692	59.5	174	583
PIPE-683	4.04	87	13.3	121	167
Compound 107	7.55	120	38.4	93.1	*n.d.*

Table 1 Pipeline compounds are potent and selective for human M1 in an mAChR recombinant membrane binding assay.

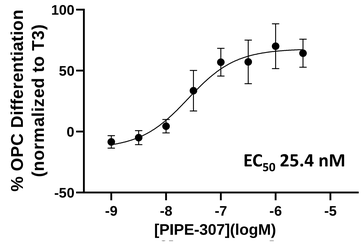
*Small molecule M3 selective antagonist from Banyu (Sagara et al 2006).
Ki (nM) for M1: 4670, M2: 6730, M3: 25.5, M4: 3600 in 3HNMS membrane binding

Calcium mobilization

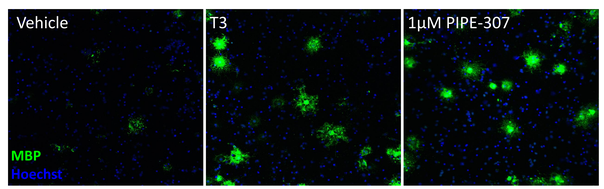
Compound	M1 IC50 (nM)	Fold selectivity against M1		
		M2/M1	M3/M1	M4/M1
Benztropine	3.19	16.9	11.2	4.78
Compound 57	0.716	343	763	430
PIPE-359	1.69	102	43	26
Compound 77	2.1	98.8	1270	212
PIPE-307	2.35	555	64.2	54.2
Compound 29	6.69	57.4	347	91.9
PIPE-683	7.45	698	175	292
Compound 107	8.91	178	117	313
Compound 51	13.5	417	1590	24.5
Compound 25	19.6	128	199	241
Compound 14	51.5	124	217	58.4

Table 2 Pipeline compounds are potent and selective in a cellular setting. Compounds were evaluated in CHO-K1 cells overexpressing one of M1-4 receptors for inhibition of ACh-induced calcium release at EC_{80} concentrations.

Rat OPC differentiation



Compound	EC50 (nM)
PIPE-683	18.9
PIPE-359	20.3
PIPE-307	25.4
Compound 107	78.5
Compound 77	175
M3 selective*	> 3.00E+03



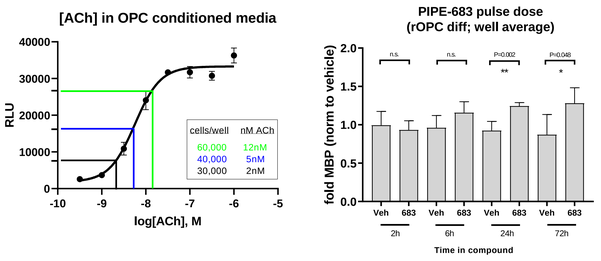
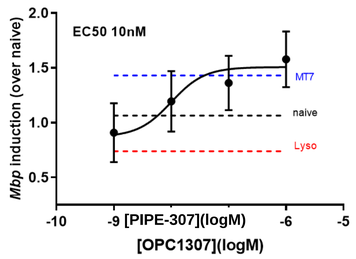


Figure 1 Pipeline compounds induce OL differentiation in rat OPCs at nM potencies. Compounds were evaluated by immunocytochemistry in rat OPCs (Mei et al 2016). ACh levels in OPC conditioned media measured by calcium flux in hM1-CHO. Pulse dosing using PIPE-683, a structural analog of PIPE-307, shows 6h exposure is sufficient to initiate OPC differentiation.

Lysolecithin mouse brain slice



Compound	EC50 (nM)
PIPE-359	7.9
PIPE-307	10
Compound 107	30
PIPE-683	36.2
Compound 77	44.3

Figure 2 Pipeline compounds induced *Mbp* in cultured cortical mouse brain slice demyelinated with lysolecithin. Slices were cultured at postnatal day 17, demyelinated and treated with compound. *Mbp* was measured by quantitative PCR. The highly M1 selective peptide MT7 was used as a positive control.

Rat cortical myelination

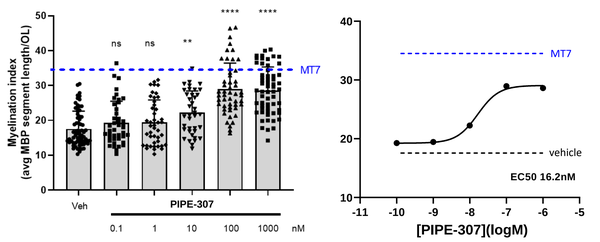
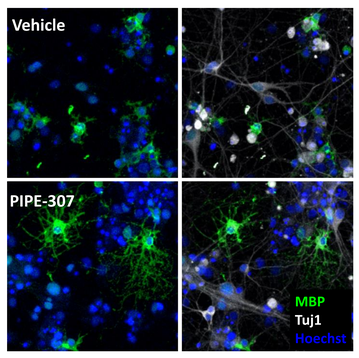


Figure 3 Differentiated OLs are myelination competent. Myelination was evaluated in a rat cortical myelination assay as described previously (Lariosa-Willingham et al 2016). Myelin segments were identified by MBP colocalization with Tuj1 (axonal marker) and averaged per OL.



Human brain slice

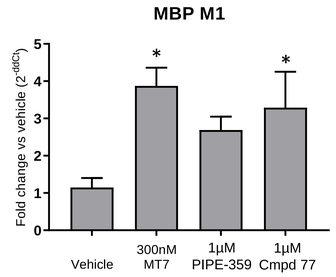


Figure 4 Pipeline M1 antagonists induced *Mbp* in a naïve human cortical brain slice assay. Slices were incubated in MT7 or compound for 9 days prior to RNA isolation and QPCR.

Dunnett's multiple comparisons test	Significant?	Summary	Adjusted P Value
Vehicle vs. MT7	Yes	*	0.0136
Vehicle vs. PIPE-359	No	ns	0.1802
Vehicle vs. Compound 77	Yes	*	0.0444

Conclusion

Selective inhibition of M1 results in the differentiation of OPCs into mature oligodendrocytes. Here, we described the identification of potent, selective small molecule M1 antagonists as evaluated by [^{3}H]NMS binding and calcium mobilization assays and further showed that these molecules induce myelination-competent oligodendrocytes. These molecules also induced *Mbp* in mouse and human organotypic slice models. Together, this provides compelling evidence that inhibition of M1 with small molecule antagonists developed at Pipeline have a positive impact in treating demyelinating disorders such as multiple sclerosis. At this point, a clinical development candidate has been identified and IND-enabling studies have been initiated.

References

Sagara, Y. et al. Identification of a novel 4-aminomethylpiperidine class of M3 muscarinic receptor antagonists and structural insight into their M3 selectivity. *J Med Chem*, 2006;*49*(19), 5653–5663.

Lariosa-Willingham, K.D., et al. Development of a central nervous system axonal myelination assay for high throughput screening. *BMC Neuro*, 2016;*17*(6).

Mei, F. et al. Accelerated remyelination during inflammatory demyelination prevents axonal loss and improves functional recovery. *eLife*, 2016; 5: e18246.