Discovering potent NMDA receptor antagonists, from the initial design stage to *in vivo* testing

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1. INTRODUCTION AND OBJECTIVES

ACTIVATION
Ca^{2+} influx
Learning
Memory

NMDAR

HYPERACTIVATION
Neuronal death
Alzheimer's disease

ISSUE

ALZEIMER'S DISEASE
- Cannot be prevented, cured or even slowed
- Alarming proportions worldwide
- **Memantine** (voltage dependent uncompetitive antagonist) **the only NMDA blocker approved for AD treatment**.[1]

SOLUTION

Synthesis, pharmacological and electrophysiological characterization of novel NMDAR channel blockers

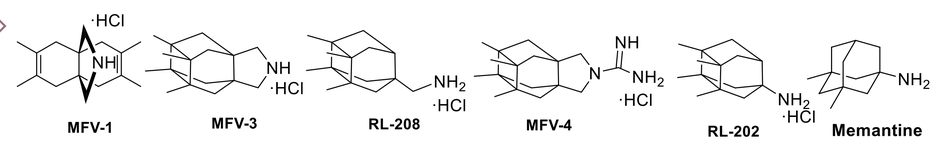
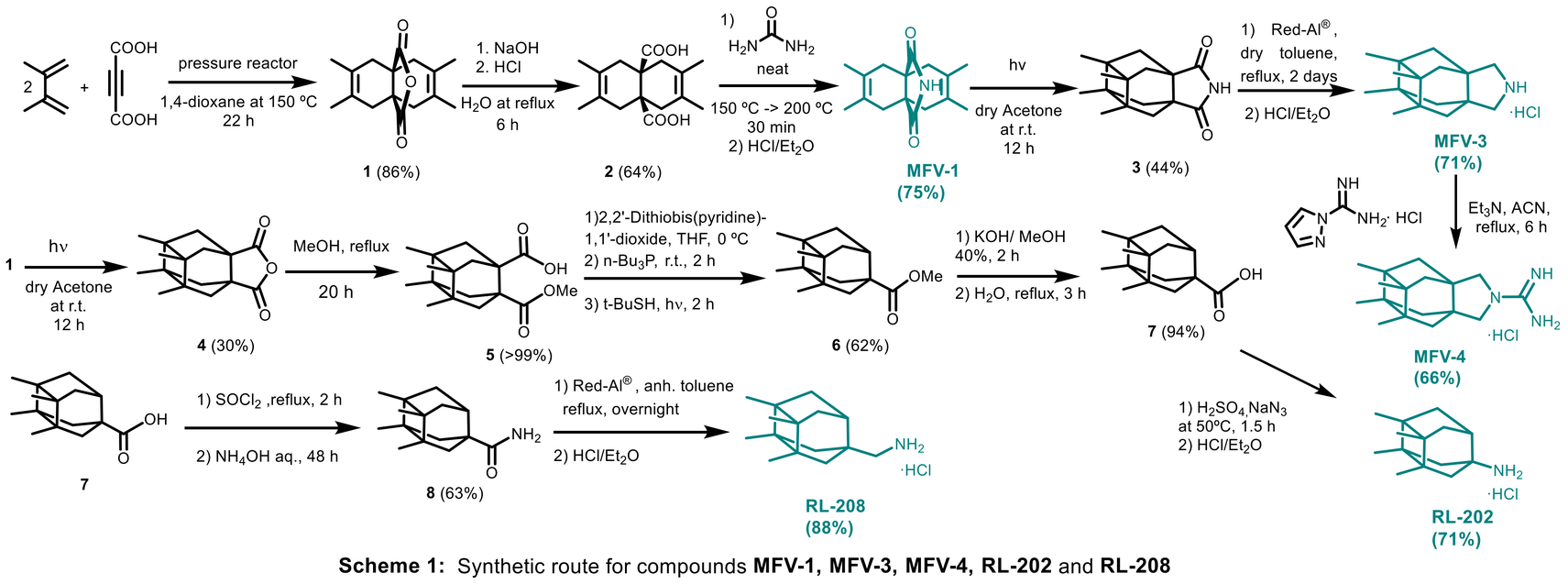


Figure 1. Memantine and the new polycyclic amine with putative NMDAR antagonist activity.

2. CHEMISTRY SYNTHESIS



Scheme 1: Synthetic route for compounds **MFV-1, MFV-3, MFV-4, RL-202** and **RL-208**

3. PHARMACOLOGY

Compound	NMDA IC_{50} (µM)
MFV-1	5.8 ± 0.96
MFV-3	5.1 ± 0.96
MFV-4	**2.7 ± 0.37**
RL-202	4.1 ± 1.69
RL-208	**2.8 ± 1.07**
Memantine	1.5 ± 0.1

Table 1. Table with the IC_{50} values of the new compounds. Ratiometric dye *FURA-2* assay was used to measure the increase in intracellular calcium in rat cerebellar granular neurons culture.

4. ELECTROPHYSIOLOGY

All compounds **exhibit comparable and similar kinetics profile** to the voltage dependent antagonist memantine (it does not block at +60 mV) used as a reference, **while MFV-4 blocked more efficiently but with some voltage independence**. Furthermore, **RL-208 exhibited faster blocking kinetics than memantine**, making it the best candidate for additional *in vitro* studies.[2]

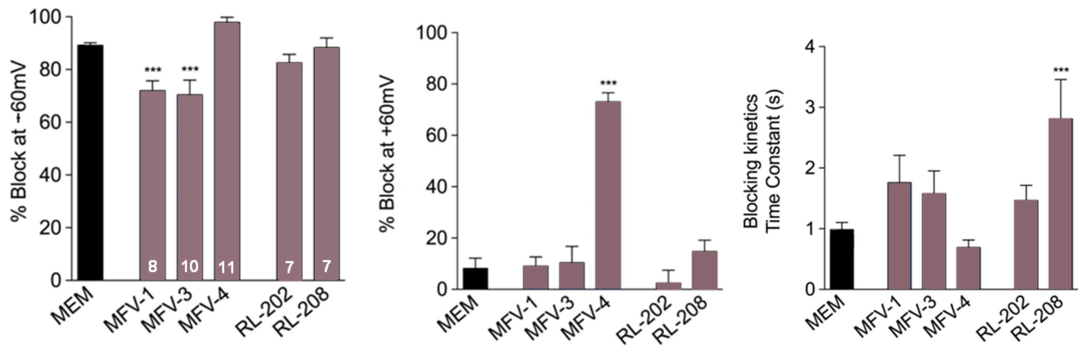
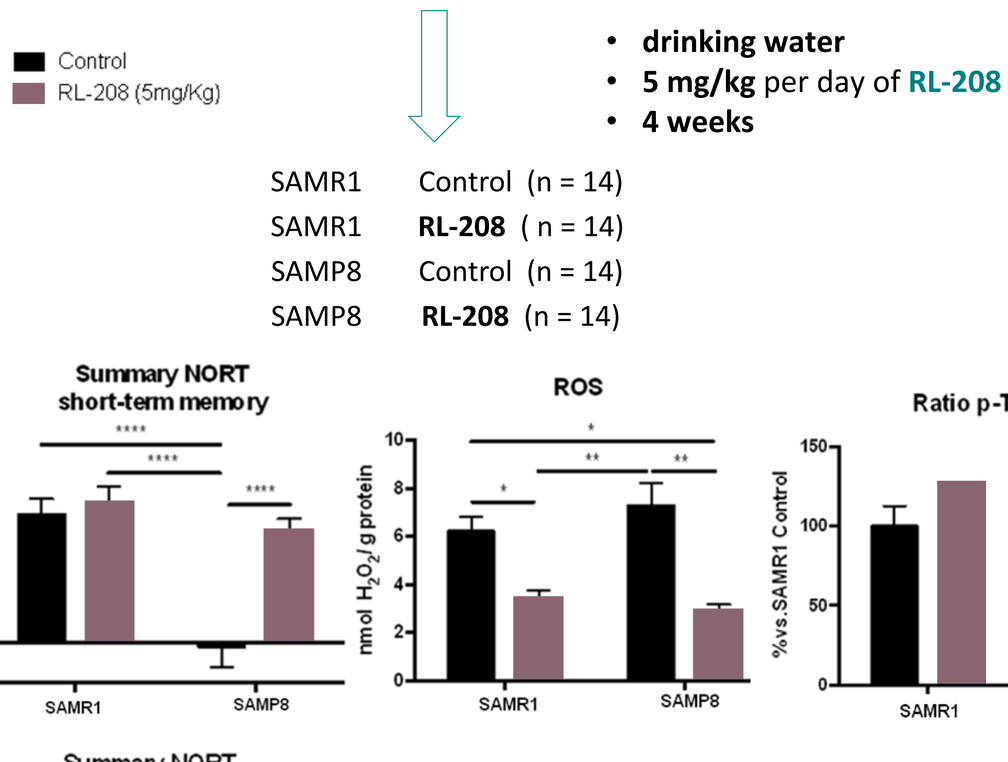


Figure 2. Whole-cell patch clamp experiments. Summary of block at -60 and +60 mV, blocking kinetics. One-way ANOVA with Dunnet comparison test. Numbers in bars denote experiments. The experiments were done in tsA201 cells expressing NMDARs (GluN1/GluN2A).

5. *IN VITRO* STUDIES AND *IN VIVO* EFFICANCY

RL-208	Microsomal stability[a]		Cytochrome inhibition[b]				hERG channel inhibition[c]
	Rat	Mice	CYP1A2	CYP2C19	CYP3A4 (BFC)	CYP3A4 (DBF)	
	66	73	5 ± 2	23 ± 1	35 ± 4	3 ± 1	50 ± 7

Tabla 2. [a]% of remaining compound after 60 min. [b]% inhibition of selected cytochromes at 10 µM concentration. [c]% inhibition of hERG channel at 10 µM.



- **Better cognitive performance** in the treated SAMP8.
- **Decrease in hydrogen peroxide** levels in both treated groups (decrease of oxidative stress).
- **Decrease in protein levels** of a neuropathologicalmarker such as **Tau hyperphosphorylation.**[3]

6. CONCLUSIONS

Five compounds have been prepared and evaluated as **novel NMDAR inhibitors** showing **comparable potencies and similar blocking** to that of **memantine.**

Oral administration of **RL-208** to SAMP8 mice that presented **cognitive impairment, reversed the cognition** status to that of control mice (SAMR1).

RL-208 is a new and promising therapeutic agent for brain disorders and age-related neurodegenerative diseases.

References

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