A Novel Series of GlyT-1 Inhibitors for Treating Schizophrenia: Is Binding Competitively with Glycine Important?

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Background

Schizophrenia is a devastating mental illness that afflicts over 1% of the world’s population. Activity of the typical and atypical antipsychotics is attributed to dopamine D2 antagonism and these agents are efficacious in ameliorating the positive symptoms, but generally are ineffective against negative symptoms and cognitive deficits of the disease. A safe and effective treatment for the entire spectrum of symptoms remains a critical unmet medical need. Emerging evidence has pointed to the glycine transporter-1 (GlyT-1) as a promising novel target for treating schizophrenia based upon the NMDA hypofunction hypothesis. GlyT-1 expression is predominantly co-localized with NMDA receptors in the forebrain. Therefore, inhibition of GlyT-1 would elevate intra-synaptic glycine levels in proximity to NMDA receptors and is anticipated to counteract the reduced response of the NMDA receptor to glutamate in the schizophrenic patient potentiating the glutamatergic neurotransmission. We surmised that a compound that bound competitively would be surmountable by increasing the synthesis rate by feeding into the transporter. The GlyT-1 inhibitor approach has been validated in a variety of pre-clinical models predictive of anti-psychotic activity and in recent human clinical trials, providing compelling evidence that GlyT-1 inhibitors show promise as a novel class of antipsychotic agents. Herein, we report the in vitro and in vivo pharmacology of AMR-GLY-6, a representative compound in a series of novel GlyT-1 inhibitors that AMRI has identified.

2. AMR-GLY-6 is Competitive for Glycine Binding Site

In addition to the orthosteric glycine binding site, GlyT-1 contains an allosteric site responsive to sarcosine-based inhibitors. We provided that, based on the intrinsic self-regulating mechanism, competitive inhibitors of GlyT-1 will have an improved therapeutic window with less mechanism based side effects. To determine the binding site for AMR-GLY-6, competitive binding assays were conducted where GlyT-1 expressed HEK-293 cells expressing GlyT-1 were exposed to increasing concentrations of [3H]-glycine in the presence of varying concentrations of compound. (A) When NFPS was tested in this assay, the maximum amount of glycine taken up decreased with increasing concentration of compound, indicating that NFPS interacts with the transporter non-competitively with the glycine binding site consistent with literature data. (B) In contrast, increasing concentrations of AMR-GLY-6 had no effect on Bmax, while the dissociation constant (Kd) increased indicative of competitive binding with glycine at the orthosteric site. We postulate that, based on the intrinsic self-regulating mechanism, competitive inhibitors of GlyT-1 will have an improved therapeutic window with less mechanism based side effects.

3. AMR-GLY-6 ADMET Profile

Pharmacokinetics

Solubility = 17µM in PBS %PPB = 87 (Human) and 92 (Rat) 
Cmax (IP) = 317µM in PBS 
Cmax (IV) = 2.25µM in PBS 
Cmax (Rat PK) = 0.833h 
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Safety

Non-Cytotoxic (EC50 = 100µM) 
Off-Target Selectivity Panel (69 targets): Clean at ≤8µM 
CYP Inhibition IC50 Values (µM): 1A2 > 100, 2B6 > 100, 2C9 > 100, 2C9 = 11, 2C9 = 197, 2D6 > 100, 3A4 = 3.3
hERG IC50 Value (nM) = 100 (PatchXpress) 
Mini-Ames = Negative

In order to progress AMR-GLY-6 to in vivo testing, we determined the ADMET and in vivo safety profile. HLM, human liver microsomes; RLM, rat liver microsomes; hERG and Selectivity Panel data provided by Recro Reserachers. Rat PK data provided by Xenometrics, LLC.

6. AMR-GLY-6 vs NFPS and Clinical Comparators

Currently, there are two GlyT-1 inhibitors in clinical development that are non-competitive for the glycine binding site. (A) Compared to these two clinical compounds, AMR-GLY-6 shows superior in vivo potency in inhibiting GlyT-1 and comparable to that of NFPS (ALX-5407). (B) In vivo in vivo brainer model, AMR-GLY-6 elevated CSF glycine to comparable levels as these compound counterparts at both 2.5 hr and 6hr of dose. NFPS significantly reduced motor activity starting at 5 hr which persisted to 24 hr when the animals were euthanized. No effects on activity were noted for AMR-GLY-6. • p < 0.05 vs. vehicle control. Tukey HSD Analysis. • No Determined.

Conclusions

• AMR-GLY-6 is a potent inhibitor of hGlyT-1 with excellent selectivity and good ADMET properties
• AMR-GLY-6 is competitive for the glycine binding site, whereas NFPS, ORG 25935 and RG1678 are non-competitive
• In vivo, AMR-GLY-6 induced elevated glycine levels in both the cerebrospinal fluid (CSF) and prefrontal cortex (PFC) of rats
• No behavioral abnormalities have been observed in animals treated with AMR-GLY-6 either acutely or after repeat doses
• These observations are consistent with our hypothesis that the competitive nature of our compounds will avoid the behavioral side-effects that have been reported for several non-competitive GlyT-1 inhibitors

References

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A. NFPS (ALX-5407) B. AMR-GLY-6

Inhibition of GlyT-1 elevates glycine levels throughout the CNS and these changes can be measured in the cerebrospinal fluid (CSF) of the rat or primate. The glycine biomarker model, an acute dose of AMR-GLY-6 decreased glycine levels in the CSF in a dose-dependent manner 2h after oral administration and comparable activity was observed after 6h (data not shown). In addition, a single administered AMR-GLY-6 for 5 days and the resulting glycine elevations were comparable to the single acute dose indicating no tolerance or accumulation occurred. Furthermore, no behavioral observations were noted following either the acute dose or repeat dosing and the effect on glycine levels was reversible with GlyT-1 (glutamate returning to baseline levels after 48 hr data not shown). Data provided by Covance Inc.

B. AMR-GLY-6

The cortex and limbic brain regions are centrally involved in the pathophysiology of schizophrenia with key interactions between the dopaminergic and glutamatergic neurotransmission networks. Therefore, we examined whether AMR-GLY-6 could elevate glycine levels in the prefrontal cortex (PFC). Dialysate samples from the PFC of rats treated with AMR-GLY-6 either acutely or after repeat doses were used to measure the effect of the PFC. Data provided by Covance Inc.

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