Discovery and characterization of potent biphasic α5GABA$_A$ receptor modulators

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Therapeutic drugs targeting GABA type-A chloride channel receptors (GABA$_A$Rs) are used as anxiolytics, sedatives, and antiepileptics. These drugs are allosteric positive modulators of GABA$_A$Rs that enhance GABA activity. As these drugs non-selectively target many GABA$_A$R subtypes causing unwanted side effects, subunit-specific drugs are now desirable. Positive modulators selective for α5-containing GABA$_A$Rs (α5GABA$_A$Rs) have shown promising results in treating cognitive symptoms of schizophrenia and age-related dementia, whereas α5GABA$_A$R negative modulators have proven worth as cognition enhancers, in post-stroke recovery and prevention of general anesthetic-induced amnesia, without causing sedation and anxiety. The aim of this project is to discover and characterize novel α5GABA$_A$R-selective compounds.

This was accomplished by screening a library of synthetic compounds on HEK293 cells, transfected with α5β3γ2L GABA$_A$Rs and an anion-quenchable yellow fluorescent protein (YFP), using a high-throughput fluorescence-based anion influx assay. Modulation on other GABA$_A$R subtypes (α1β2γ2L, α5β2γ2L) and drug binding sites were explored *via* functional studies using two-electrode voltage clamp electrophysiology (TEVC). To prepare for TEVC, *Xenopus borealis* frogs were anesthetized with MS-222 and surgically incised to obtain the oocytes, which were digested and injected with GABA$_A$R mRNAs.

TEVC recordings revealed that isomeric compounds RM 68, RM 69 and RM 70 are potent GABA$_A$R modulators. These isomers, except RM 69, demonstrated biphasic modulation, selectively potentiating α5GABA$_A$Rs in the nanomolar (sometimes picomolar) range, but inhibiting GABA$_A$Rs non-selectively at higher micromolar concentrations. Removing mercaptoacetaldehyde from RM 68 to give RM 96 eliminated its α5GAB$_A$AR selectivity. Flumazenil managed to neutralize the potentiation of GABA responses by the 4 compounds, suggesting that the potentiating effect of these compounds was modulated *via* the benzodiazepine site.

RM compounds can be potential leads to design novel subtype specific drugs for α5-related disorders and to study the pharmacology of α5GABA$_A$Rs.