

Novel Compounds for Anxiety, Epilepsy, and Neuropathic Pain

(OTT ID 1268)

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Problems:

- Classical benzodiazepine (BDZ) psychoactive drugs most often have major side effects including tolerance development, addiction, and adverse psychological and physical effects
- Tolerance has been attributed to the $\alpha 1$ and $\alpha 5$ GABA_A receptor subtypes
- Reports have shown that patients using BZDs for schizophrenia have a higher likelihood of early death by suicide, partly attributable to the severe anxiety and insomnia experienced as withdrawal symptoms if they stop using BZDs or take them for extended periods

Solution:

- The inventors have developed novel imidazobenzodiazepine (IMBDZ) compounds useful for the treatment of anxiety disorders, epilepsy, and neuropathic pain
- Compounds specifically target the $\alpha 2$, $\alpha 3$, or $\alpha 2/3$ subunit of the GABA_A receptor
- It is believed that agents acting selectively at these receptors would aid in preventing the side effects of sedation, ataxia, amnesia, tolerance, and abuse potential
- The lack of ester linkages in these novel ligands make them less sensitive to hydrolysis by esterases leading to greater stability in human liver microsome assays
- Preliminary studies in a rhesus monkey Vogel conflict assessment model suggests an anxiolytic effect in lead compounds
- They are stable in human blood, brain, and kidneys (CRO)

Market

- Anxiety disorders represent the most commonly occurring mental health condition in all age groups
- A Global Industry Analysts report on anxiety disorders forecast the global market to reach \$5.9 billion by the year 2017
- The market growth is attributed to the increase in stress levels worldwide related to financial crisis, increased incidence of disorders, the growing aging population, the existence of significant unmet needs, and the recent development of novel therapeutics
- The U.S. represents the largest regional market for anxiety disorder therapeutics worldwide, and costs the U.S. over \$42 billion per year accounting for almost one-third the overall cost for mental illnesses
- Anxiety disorders are highly treatable yet only about one-third of those suffering seek treatment
- The Cook ligands should be active against GAD, SAD, OCD, PTSD, and many phobias.

Intellectual Property

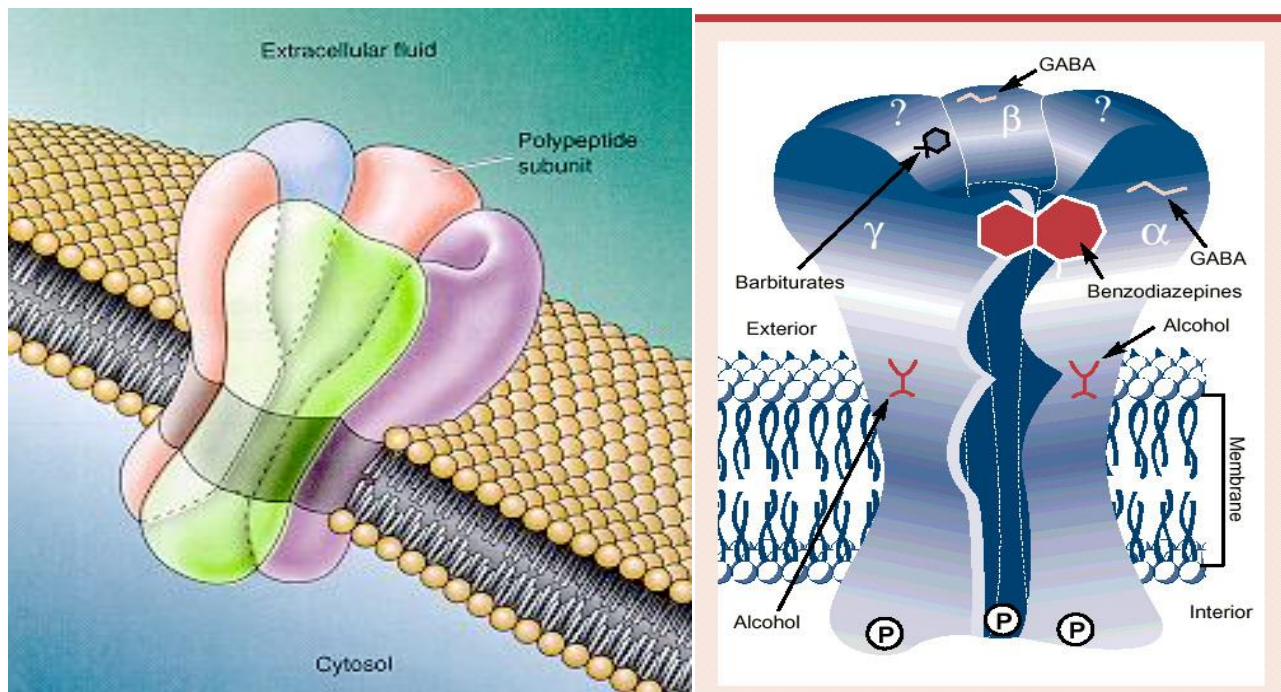
- U.S. Utility Patent Application has been filed for this technology

Partnering

- Looking for a development partner to aid in the development of a final product
- This technology is available for licensing under exclusive or non-exclusive terms

- To elucidate the role of specific GABA_A/benzodiazepine receptor subtypes in regulating anxiety and pain, a number of imadazobenzodiazepines have been synthesized and evaluated
- Tolerance to treatment has been attributed to the $\alpha 1$ and $\alpha 5$ subtypes
- The Cook ligands are nearly silent at the $\alpha 1$ and $\alpha 5$ BDZ GABA subtypes (oocyte receptor binding assays), therefore promoting anxiolytic and anticonvulsant effects devoid of the side effects observed using $\alpha 1$ -subtype ligands
- The lead compounds are metabolically stable in human liver microsome assays and one compound shows an anxiolytic effect in a preliminary rhesus monkey trial.
- These compounds are designed to be more metabolically stable derivatives of an earlier lead compound that was highly effective in anxiety studies in many animal models but lacked the metabolic stability necessary for further clinical trials

GABA/BzR chloride ion complex



- The benzodiazepine receptor (BzR) binding site is an allosteric modulatory site located on the GABA(A)ergic ion channel shown above.
- It is located between the α and γ subunits, while the true neurotransmitter site, GABA, is located between the α and β subunits of this pentameric protein complex.
- BzR are allosteric, therefore, the chances of side effects should be decreased as compared to agonists or antagonists at the GABA receptor

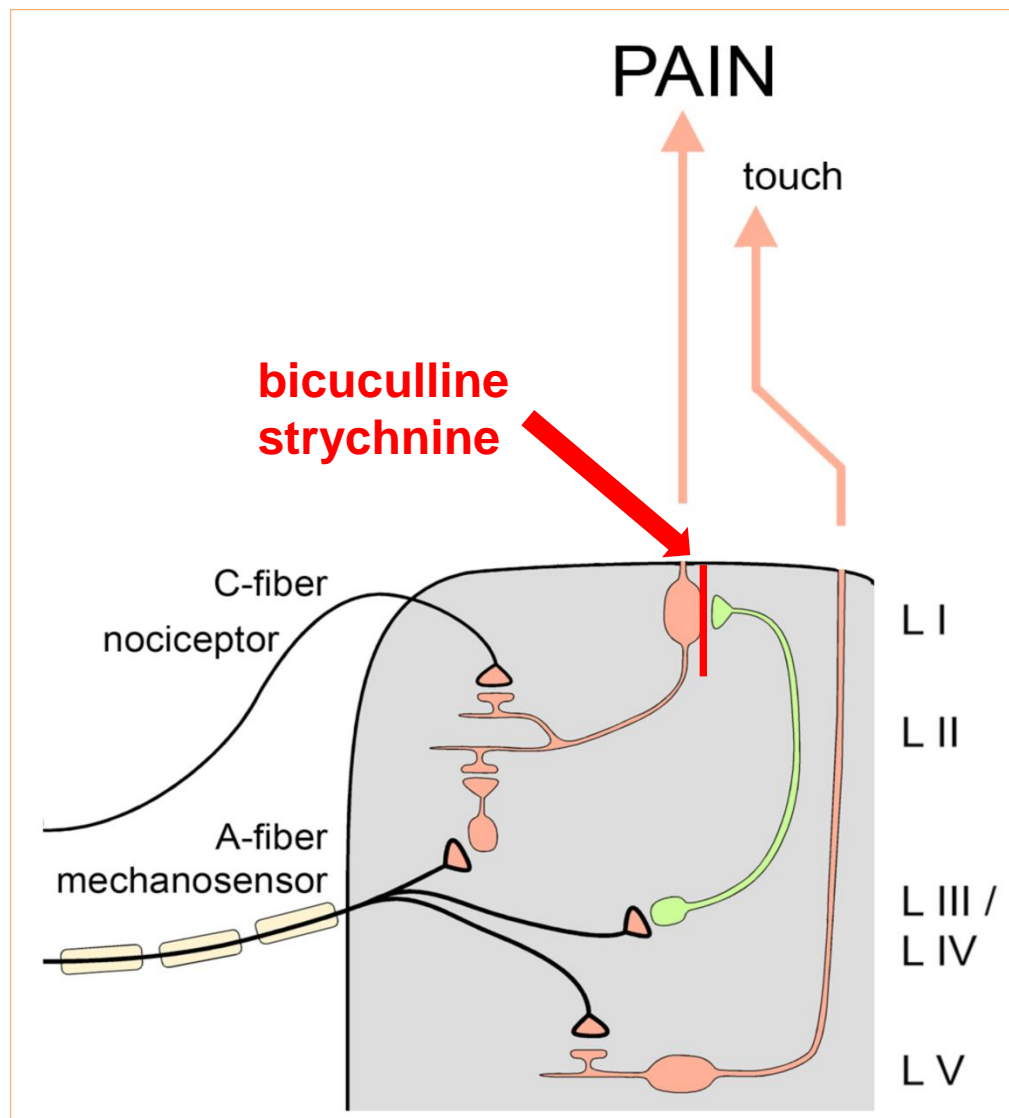
From *Neuroscience: Exploring the Brain*, Bear, MF, Connors, BW and Paradiso, MA; William & Wilkins, Baltimore, 1996.

Subunits	Associated Effect
α_1	Sedation, anterograde amnesia, some anticonvulsant action, ataxia, some addiction
α_2	Anxiolytic, hypnotic (EEG), maybe some muscle relaxation at higher doses, some anticonvulsant action
α_3	Some anxiolytic action, some anticonvulsant action, maybe some muscle relaxation at higher doses
α_4	Diazepam-insensitive site
α_5	Cognition, temporal, and spatial memory, (Maybe memory component of anxiety)
α_6	Diazepam-insensitive site

Blockade of inhibitory neurotransmitters induces

- hyperalgesia
- allodynia (touch-evoked pain)
- spontaneous activity of dorsal horn neurons (spontaneous pain)

- Data by Yaksh et al. and others have shown that pharmacological blockade of spinal GABAergic or glycinergic inhibition induces hyperalgesia (increased sensitivity to painful stimuli) and allodynia (painful sensation of stimuli which are normally not sensed as painful) in animals.
- Electrophysiological work has shown that the blockade of glycine and GABA receptors dis-inhibits polysynaptic connections from touch sensitive fibers to normally pain-specific projection neurons and induces spontaneous activity in these cells.
- Blocking dorsal horn glycine and GABA receptors therefore mimics many symptoms characteristic of chronic pain in humans.

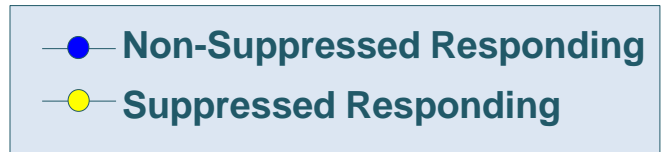
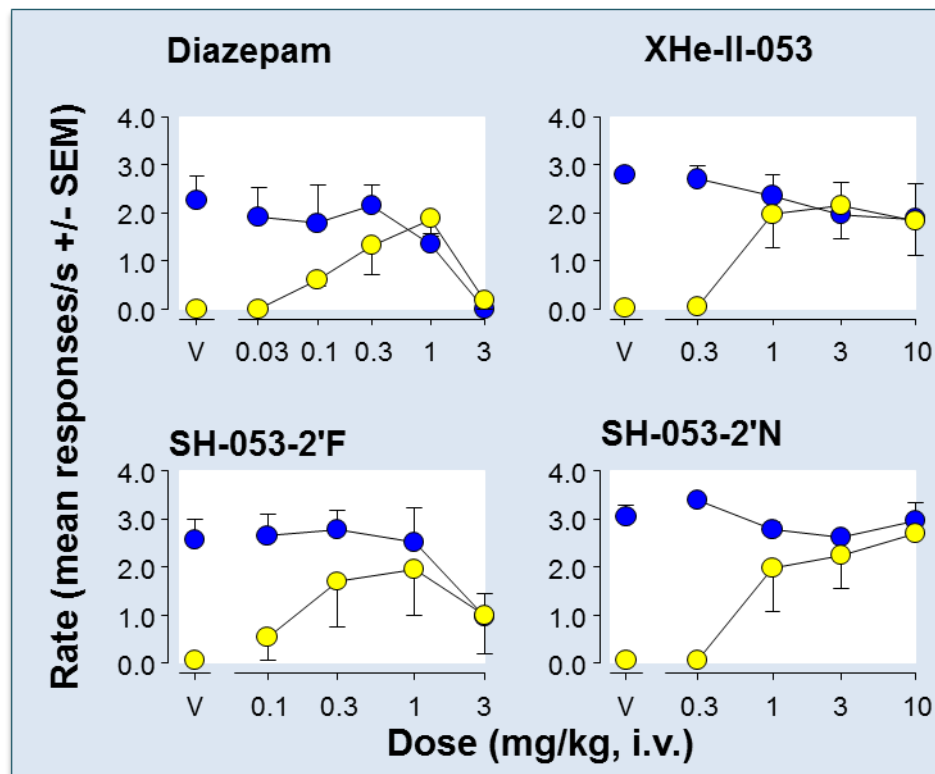


- The Cook lab created several novel compounds targeted to the α_2 , α_3 , or $\alpha_2/3$ subunits of the GABA_A receptor
- XHE-II-053 was formerly tested in dogs and primates but lacked stability in humans in Phase I trials
- HZ-166 (SH-053-2'N) is a stable derivative that was effective in treating anxiety in rodents and rhesus monkeys with a patent life that ends in 2022
- A utility patent application filed this year (Priority April 2011) with the UWMRF covers novel stable compounds related to these former leads
- The Cook lab is now working with collaborators to collect further biological data in anxiety, pain, epilepsy, and schizophrenia models

- The figure shows the effects of compounds on responding maintained by food in the absence (Non-Suppressed Responding) and presence of response-contingent shock presentation (Suppressed Responding).

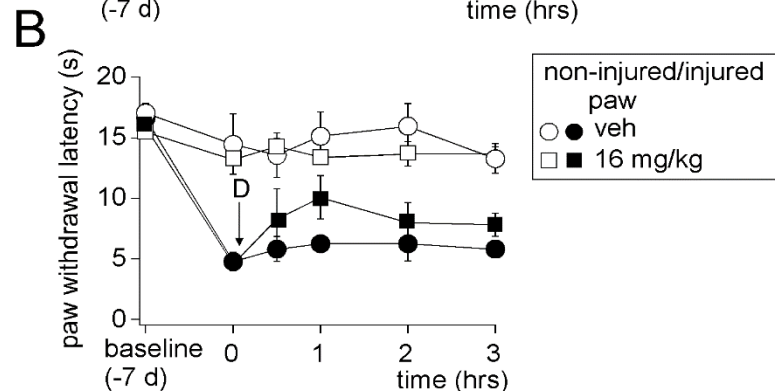
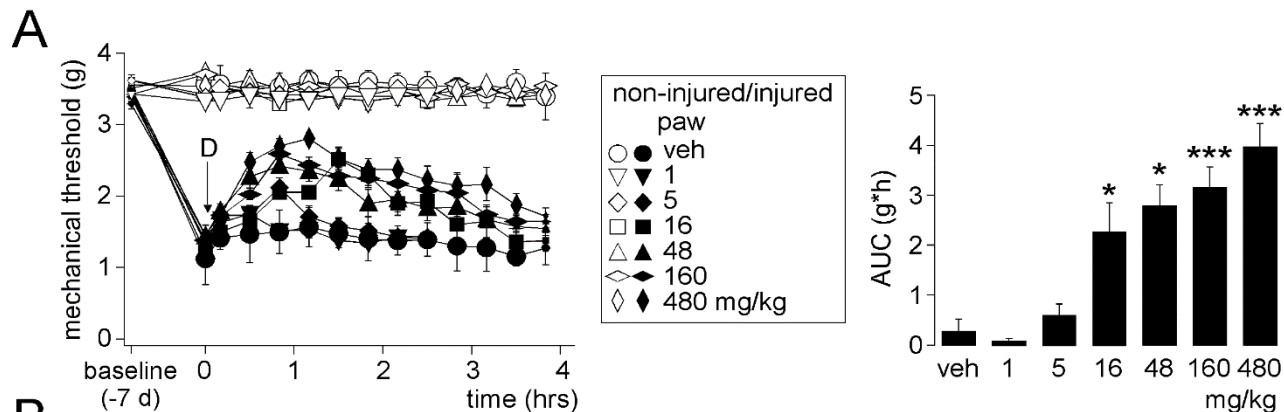
- An increase in Suppressed Responding indicates an anxiolytic-like effect, while a decrease in Non-Suppressed Responding indicates a non-specific effect on responding (e.g. sedation, motor impairment)

- XHe-II-053 and HZ-166 (2'N) had an anxiolytic-like effect with no non-specific effect.

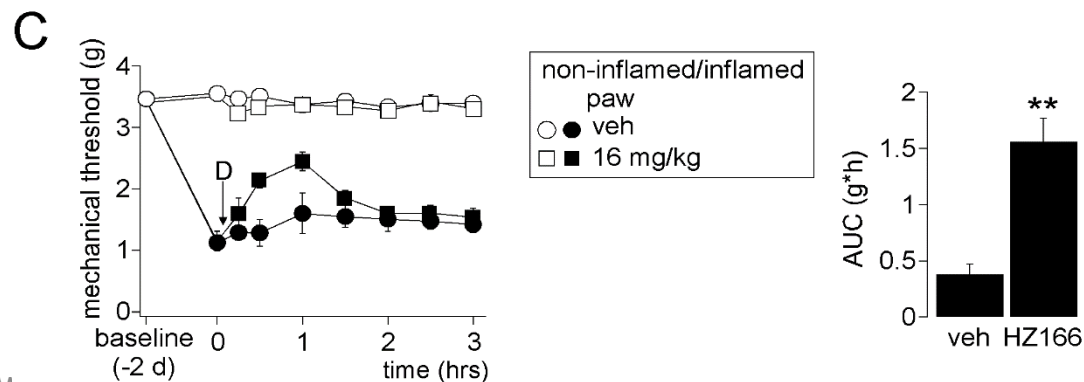


HZ166 reduced Neuropathic and Inflammatory Pain in Rats

Neuropathy



Inflammation



- In the chronic constriction model, mice treated with HZ-166 (A, B) experienced less neuropathic pain and less inflammatory pain (C)

- In addition, this work was repeated in the sciatic nerve ligation model at NINDS with the same result in A and B as well as by a CRO with the same result; tolerance did not develop after 14 days

Di Lio, et al.

- A library of new compounds were designed to be more stable than HZ-166 and XHE-II-053
- The lack of ester linkages in these novel ligands make them less sensitive to hydrolysis by esterases leading to greater stability
- The compounds were tested using radioactive in vitro binding and competition assays, electrophysiological *Xenopus* oocyte recordings for the GABA receptor, and in human liver microsome stability assays
- One compound has also been tested preliminarily in rhesus monkeys

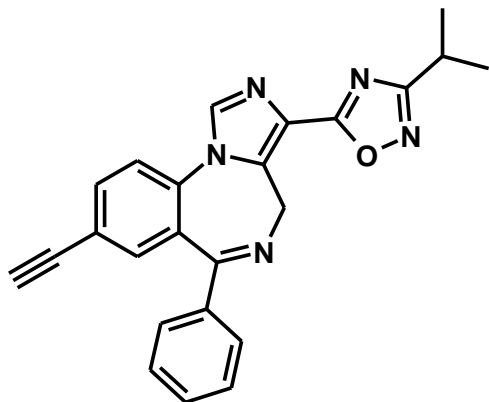
Competitive Binding Assays

Compound	α_1	α_2	α_3	α_4	α_5	α_6
EMJ-I-026	5000	135	1027	ND	152	5000
JY-1-59	1.08	2.6	11.82	ND	11.5	5000
DM-II-20	54.3	27.14	35.68	ND	15.3	5000
ZJW-II-040	4753	25.54	31.43	ND	275.4	ND
YT-III-15	73.19	90.45	141.4	ND	114	ND
HJ-I-037	22.16	44.06	38.48	ND	12.15	ND
YT-III-31	36.39	67.85	129.7	ND	7.59	ND
YT-III-271	32.54	1.26	2.35	ND	103	ND

- Lower numbers predict better binding at the site
- See next slides for actual binding results in oocytes

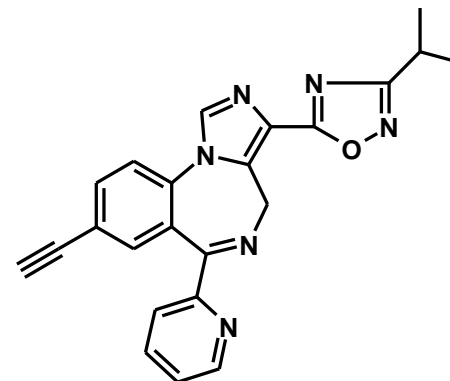
The assays were performed in a total volume of 0.5 mL at 4 °C for 1 hour using [³H]flunitrazepam as the radiolabel. For these binding assays, 20-50 mg of membrane protein harvested with hypotonic buffer (50 mM Tris-acetate pH 7.4 at 4 degree) was incubated with the radiolabel as previously described (Choudhary et al. *Mol Pharmacol.* 1992, 42, 627-33; Savić et al. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 2010, 34, 376–386)

EMJ-I-026

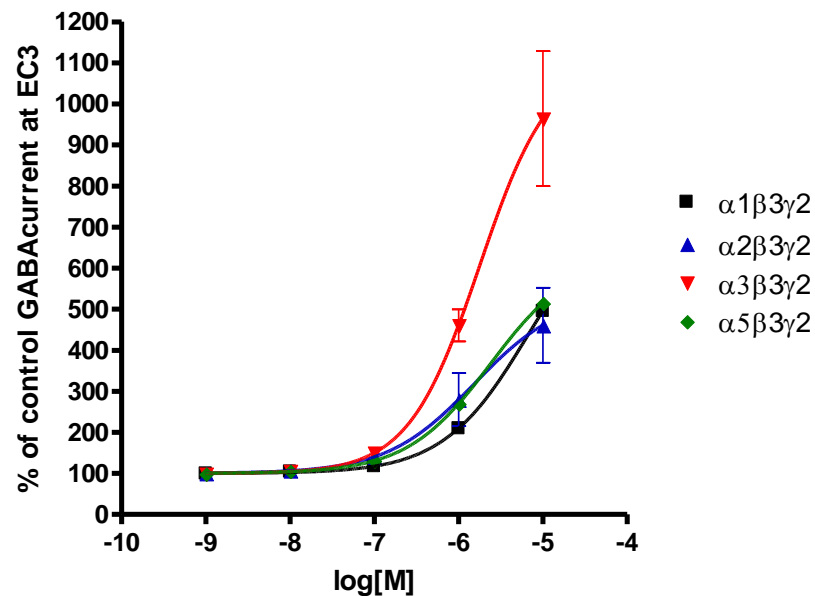
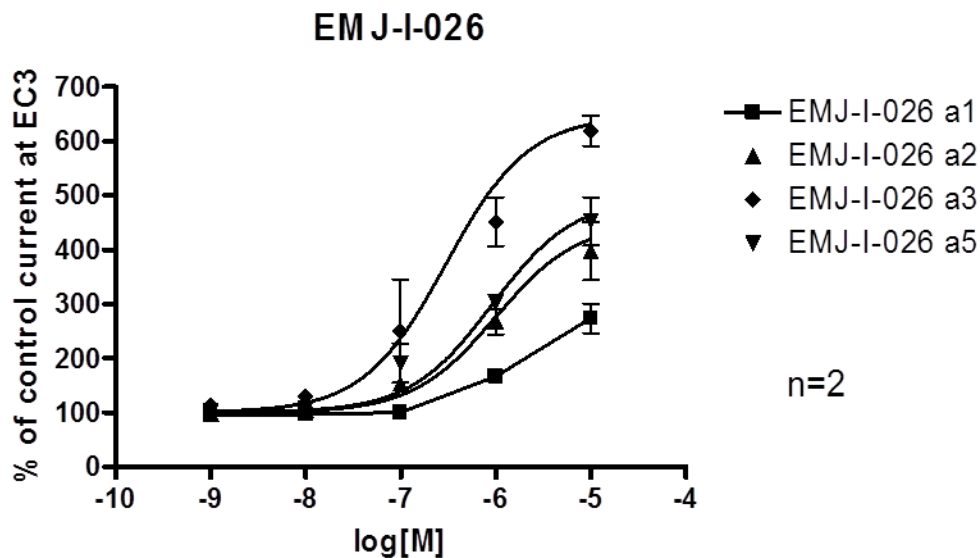


***Both compounds are $\alpha 3$ targeted**

ZJW-II-40



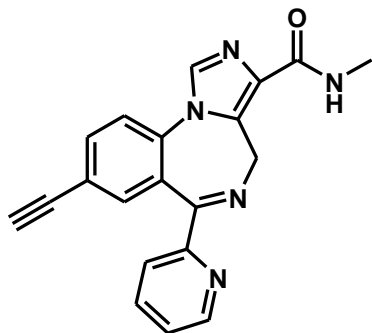
ZJW-II-40



EC3: A concentration of GABA eliciting 3% of the maximal GABA-elicited current amplitude of the individual oocyte.

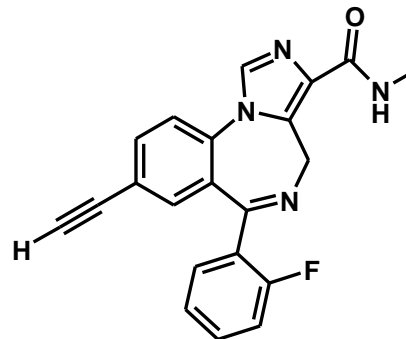
Concentration Effect Curves: Electrophysiological recordings in oocytes

HJ-I-040

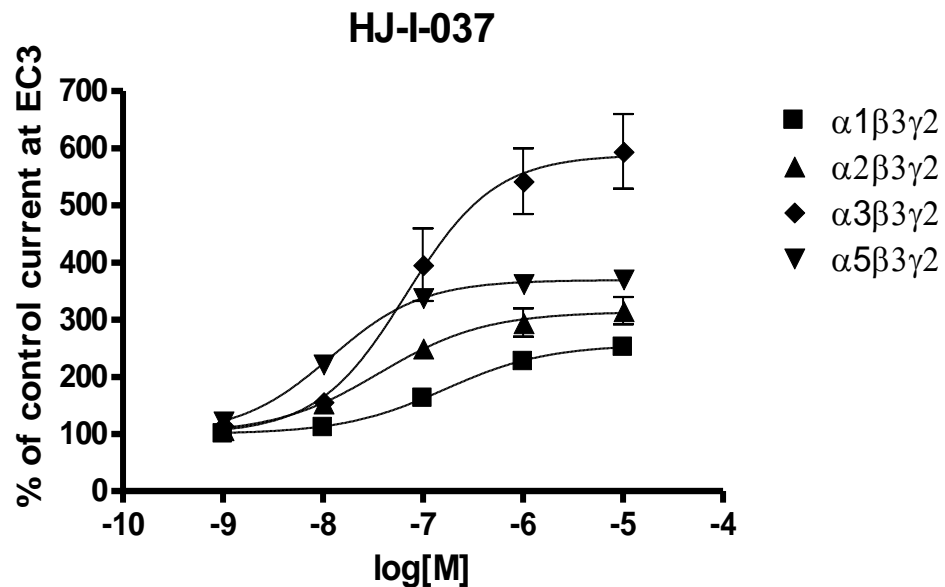
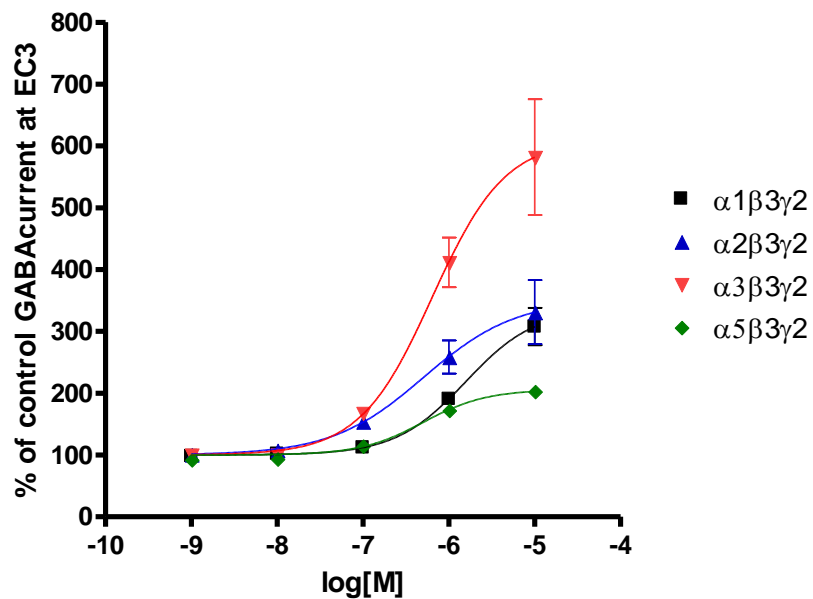


HJ-I-40

HJ-I-037

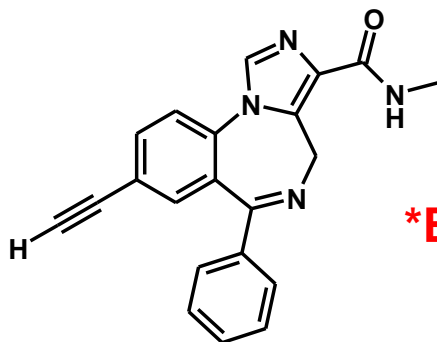


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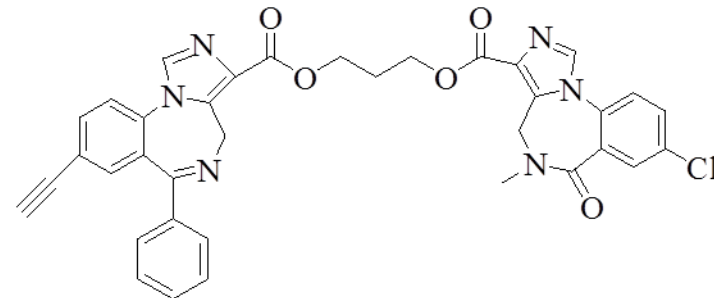
Concentration Effect Curves: Electrophysiological recordings in oocytes

YT-III-31

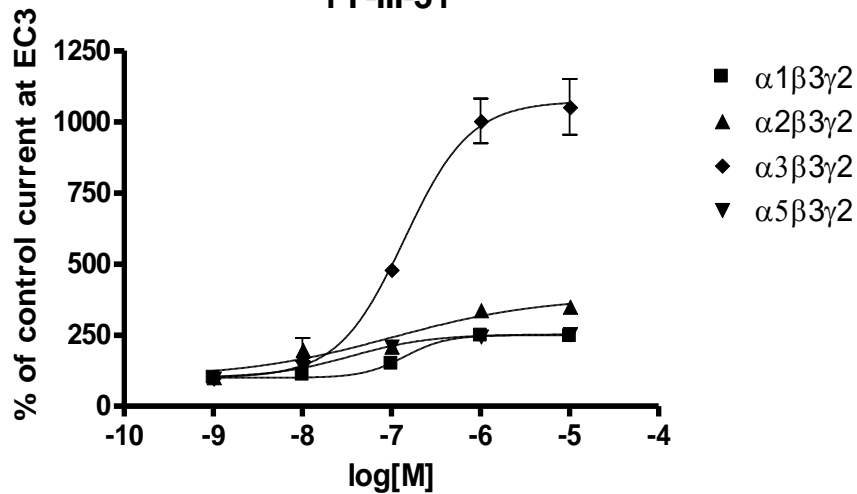


***Both compounds are $\alpha 3$ targeted**

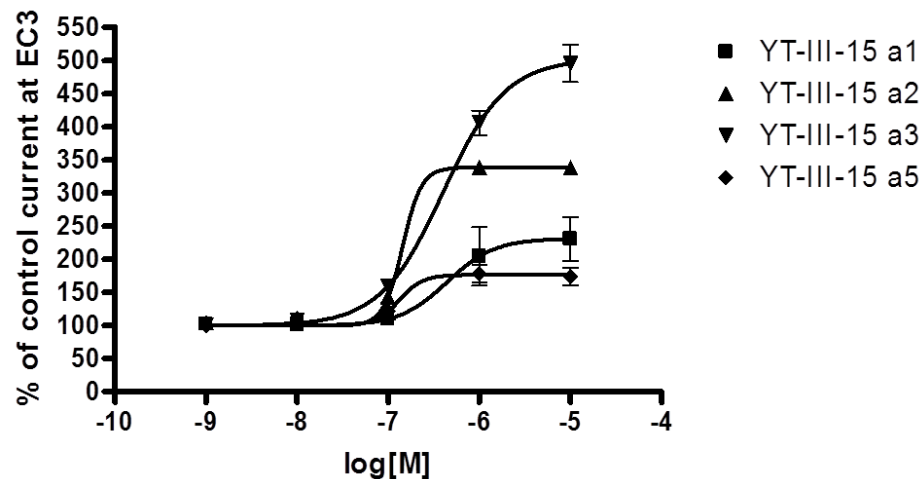
YT-III-15

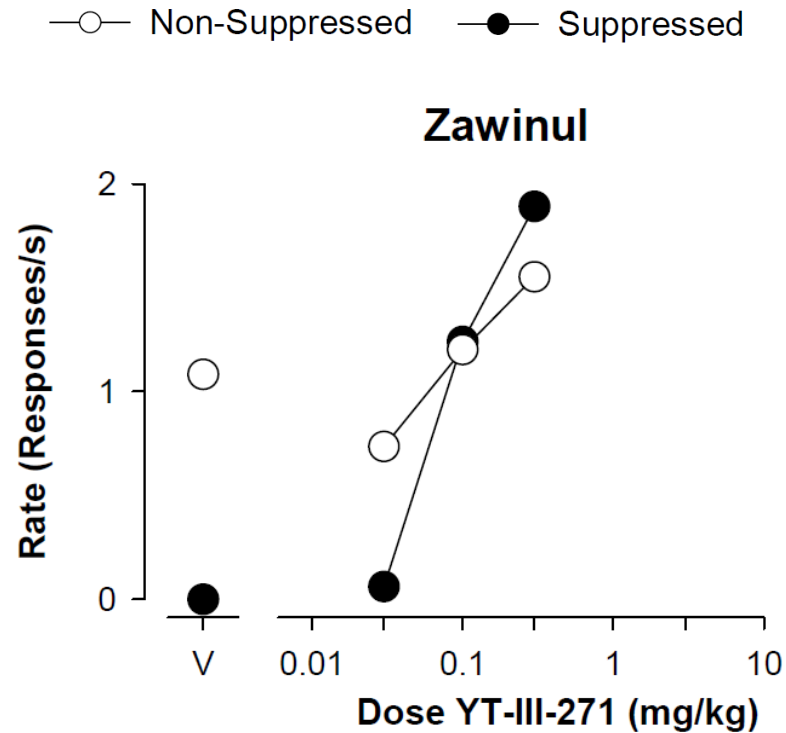


YT-III-31



YT-III-15_ZV





- Figure shows the Vogel conflict assessment of anxiolytic and sedating effects in a rhesus monkey as described in slide 9
- Preliminary results show an anxiolytic effect for YT-III-271

Compound	Stability Results
XHE-II-053	Significant metabolism at 1 and 10 μM
HZ-166	Slight metabolism at 1 and 10 μM
EMJ-026	Not metabolized at 1 and 10 μM
ZJW-040	Not metabolized at 1 and 10 μM
HJ-I-037	Not metabolized at 1 and 10 μM
HJ-I-040	Not metabolized at 1 and 10 μM
YT-III-15	Significant metabolism at 1 μM and slight metabolism at 10 μM
YT-III-271	Slight metabolism at 1 μM and no metabolism at 10 μM

- The metabolic stability of GABA_A receptor ligands using human liver microsomes was studied. The test articles were incubated at two concentrations (1 and 10 μM) and aliquots (100 μl) were removed at various time points (0, 15, 30 and 60 minutes), and analyzed by LC-MS/MS.

- Novel stable imadazobenzodiazepine analogs have been designed to specifically bind to the $\alpha 2$ or $\alpha 3$ subunits of the GABA(A) receptor
- Compounds of this type could prove useful in the treatment of anxiety, neuropathic pain, and epilepsy
- Liver microsome assays show that several of the new compounds are stable
- *In vitro* binding assays and electrophysiological recordings show the specificity of several of the compounds for the $\alpha 3$ subtype
- Some preliminary data shows promise in a rhesus monkey anxiety model

Further investigations required

- Testing of the compounds with collaborators in rats assays designed for models of:
 - Drug tolerance
 - Schizophrenia
 - Pain
 - Anxiety
 - Epilepsy
- Find a partner to aid in conducting further animal trials, pre-clinical, and clinical testing

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