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## **Title: Towards Subtype-Selective Modulators of GABAA Receptors**

GABA (*g*-aminobutyric acid) is the most abundant neurotransmitter in the mammalian nervous system (Macdonald and Olsen, 1994). In the adult nervous system, GABAA receptors mediate the fast inhibitory effects of GABA, while slow inhibition is mediated by GABAB receptors. GABAA receptors are GABA-gated chloride channels and they are the site of action of many clinically important drugs (Sieghart et al., 2012).

Barbiturates, neuroactive steroids, general anesthetics, and benzodiazepines, are some of the modulators that act via GABAA receptors (D'Hulst et al., 2009). Many of them modulate all GABAA receptors with similar potency and efficacy and this lack of selectivity for certain receptor subtypes leads to unwanted side effects. Thus searching for subtype selective ligands is a major goal in drug development. The majority of GABAA receptors consists of two *a*, two *b*, and one *g* subunits. The GABA binding sites are located at the extracellular *a-b+* interface (Smith and Olsen, 1995), and the benzodiazepine binding site is located at the *a+g-* interface (Sigel and Buhr, 1997). It was found recently that the modulation of the pyrazoloquinolinone CGS 9895 is mediated via a novel binding site located at the extracellular *a+b-* interface (Ramerstorfer et al. 2011).

In this thesis I investigated the effects of pyrazoloquinolinones mediated via the *a+b-* binding site of GABAA receptors. The results of this thesis have been published in two papers. The first of the two papers deals with the effects of 32 structural analogues of CGS 9895 at *a1b3* and *a1b3g2* GABAA receptors to understand whether they mediate their effects in a way similar to CGS 9895 and to identify compounds with higher potency (Varagic et al., 2013a). A quantitative structure activity relationship (QSAR) analysis for 20 of the 32 pyrazoloquinolinones and pyrazolopyridinones was then applied to the results of the electrophysiological experiments in order to understand the effects of variations in their chemical structure on the potency at the *a1+b3-* binding site. This resulted in a model that predicts the effects of substituents at two pyrazoloquinolinone key positions on potency. Another outcome of this paper was the identification of competitive null modulators for the *a1+b3-* binding site. These compounds inhibit the effects of positive allosteric modulators acting via the *a1+b3-* interface, and were used in the second paper.

In the second paper I investigated the effects of LAU 177 at GABAA receptors. LAU 177, among all compounds we had characterized at that time, showed the highest efficacy of modulation at *a1b3* and *a1b3g2* receptors and therefore this compound was chosen for further investigations. I demonstrated that this compound strongly enhanced GABA-induced currents at all GABAA receptors investigated. The extent of modulation was comparable in *ab* and *abg2* receptors but depended on the type of *a* and *b* subunits present within the receptors. I also for the first time investigated the effects of this and some other compounds on *d*-containing receptors (*a1,4,6b3d*). LAU 177, in contrast to the other pyrazoloquinolinones investigated, showed an unexpectedly high efficacy at *a1b3d* but not at *a4,a6b3d* receptors. The efficacy of LAU 177 at *a1b3d* receptors was threefold higher than at the corresponding *a1b3* and *a1b3g2* receptor subtypes. Further investigations at *a1b3d* receptors indicated that this compound not only at *a1b3g2*, but also at *a1b3d* receptors elicited most of its effects via the *a+b-* interface.

In summary we identified a super-modulator for *a1b3d* GABAA receptors and demonstrated that the efficacy of a compound not only depends on the compound structure but is also influenced by each subunit present in the receptor irrespective of its contribution to the drug binding site.