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**MEETING ABSTRACT**

**A3.4**  
**Binding behavior of different benzodiazepine ligands implies the use of more than one binding pose in their interaction with GABA<sub>A</sub> receptors**  
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**Background:** GABA<sub>A</sub> receptors are ligand-gated chloride channels, one of the major inhibitory receptors in the central nervous system. GABA<sub>A</sub>-Rs are heteropentamers made up from 19 known subunits and are targets for many clinically important drugs. Among them are the family of the widely used benzodiazepines (BZs), which bind to GABA<sub>A</sub>-Rs at the α+γ2- interface. Understanding the particular molecular interaction of BZs and their GABA<sub>A</sub>-R binding site is of crucial importance in developing new BZ ligands. It is assumed that all BZs interact with the receptor alike in a “common” binding mode, whereby three were suggested (CBM I, CBM II and CBM III). However, it is still argued in literature which is the most probable one. Some docking studies suggest CBM I, while others support CBM II. Previous docking studies in our lab suggested that a chiral methyl group, (position 3 of the 7-membered diazepine ring) could be used as a clinical reporter. Accordingly, we performed new computational modelling, which confirmed that ligands having a methyl group both in the (R)- as well as in the (S)-conformation can bind in binding pose I. If the drug, however, binds in binding pose II, only (S)-isomers will be able to bind, since (R)-isomers are sterically hindered. The aim of the current study was to test this hypothesis and provide experimental evidence in favor of one or the other binding mode.  

**Methods:** Several sterosisomeric drugs from three different structural BZ classes, namely diazepam-, imidazobenzodiazepine- and triazolam-derivatives were investigated. We used <sup>3</sup>H]flunitrazepam displacement as well as two-electrode voltage-clamp electrophysiology in recombinantly expressed GABA<sub>A</sub>-R subtypes containing α<sub>1</sub>β<sub>3</sub>γ<sub>2</sub>, α<sub>2</sub>β<sub>3</sub>γ<sub>2</sub>, α<sub>3</sub>β<sub>3</sub>γ<sub>2</sub> and α<sub>5</sub>β<sub>3</sub>γ<sub>2</sub> to determine ligand binding and functional activity of the three BZ classes.  

**Results:** Interestingly, both imidazobenzodiazepine (S)- and (R)-isomers exhibited comparable binding affinities while the other two classes displayed a dramatic difference in binding affinities. Thereby, the (R)-isomers showed complete loss of binding ability whereas the (S)-isomers remained active.  

**Discussion:** As predicted from our computational modeling, our experimental data indeed could provide insight into the nature of the interaction of different BZ with their specific GABA<sub>A</sub>- receptor binding site. Surprisingly, the tested ligands did not behave in an identical manner. According to our results, we could conclude that different chemically related benzodiazepine ligands tend to interact via different binding modes rather than using a common one.

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