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OC.2-A FLUORESCENCE-BASED ASSAY TO EVALUATE THE ACTIVITY OF Kv7.2/Kv7.3 CHANNEL MODULATORS

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Potassium channels encoded by the Kv7 subfamily are critical regulators of neuronal excitability and attractive pharmacological targets for several neuropsychiatric conditions. In particular, Kv7.2 and Kv7.3 subunits underlie most of the M-current (IKM), a sub-threshold K^+ current controlling action potential generation at the axon initial segment. Mutations in the genes encoding for Kv7.2 and Kv7.3 are responsible for early-onset epilepsies. Moreover, the Kv7.2/Kv7.3 channel activator retigabine has been approved as a novel anticonvulsant, but has been now withdrawn because of its poor selectivity for Kv7 subtypes, short half-life, poor brain penetration and chemical instability. In this work, we have: 1. developed a fluorescence-based assay to indentify novel Kv7.2/Kv7.3 channel modulators, 2. used this fluorescence assay to evaluate the activity of a small library of retigabine derivates, and 3. compared the fluorescence results with electrophysiological data. Stable CHO cell lines expressing Kv7.2 and Kv7.3 channel subunits generated by the PiggyBac Transposon System were used in a Thallium (Tl^+)-based assay with a fluorescent Tl^+-sensitive dye (FluxOR Green Potassium Ion Channel Assay). Retigabine (0.3 to 100 µM) dose-dependently increased the maximal fluorescence and the initial slope of the fluorescent signal; both effects were abolished by the Kv7 blocker XE991 (10µ M). The calculated EC_{50} for retigabine was 5.0±0.6µM, a value slightly higher than the EC_{50} calculated by electrophysiological techniques (1.9±0.2 µM). Several compounds able to increase the maximal fluorescence and the initial slope of the fluorescent signal, in some cases with higher potency and efficacy when compared to retigabine, were identified.