Discovery of CP86, a potent neuronal Kv7 channel activator with in vivo anticonvulsant effects

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Kv7 K^+ channels play a pivotal role in controlling neuronal excitability, representing attractive pharmacological targets for the treatment of different neurological disorders, including epilepsy. In fact, the Kv7.2/7.3 agonist retigabine has been approved in 2011 as add-on treatment for adults with drug-resistant partial onset seizures with or without secondary generalisation. However, due to several drawbacks and side effects, the clinical use of retigabine has been declining over the years, leading to its discontinuation in 2017.[1] In the attempt to overcome some of these limitations we started from our previously published results[1] to perform in-silico studies of the retigabine binding site. The homology models generated allowed the identification of a putative non-explored, wide, lipophilic pocket at the bottom of the pore of the channel. This pocket was explored by a specifically designed molecular library of 41 retigabine derivatives. Site-specific mutagenesis experiments validated the binding site and the compounds binding pose, involving an extended interaction network with W236, V225, F240, S303, F304, F305 and L312, in the Kv7.2 subtype homotetrameric assembly. Some of the predicted interactions are in accordance with literature data[2] and have been confirmed by the recently released cryo-EM structure of the Kv7.2 subtype.[3] In addition, our results further expand the structure-activity relationship clues for the rational design of Kv7 channels agonists, highlighting the importance of the newly identified lipophilic pocket in drugs-target interaction. The synthesized compounds, were characterized by a Tl^+-based fluorescent HTS assay; the most potent compounds were also studied with patch-clamp electrophysiology. When compared to retigabine, the newly-synthetized compounds showed a more marked leftward shift of the current activation curve, and an enhanced maximal currents. The most potent compounds were also subjected to an extensive in vitro and in vivo preclinical characterization leading to the identification of CP86 as a metabolically stable derivative, with a remarkable CNS distribution. CP86 shows a 4-fold increase in brain concentration and a 80-fold increase in brain-to-blood ratio when compared to retigabine. Moreover, as assessed by photostability experiments, the compound does not generate the toxic photo-induced metabolites, that are responsible for retigabine precipitation in light-exposed tissues, including the retina. Finally, when tested in vivo in the pentylenetetrazol (PTZ) model of acute seizures, CP86 (0.1-1 mg/kg) significantly reduced the latency and severity of PTZ-induced seizures; these effects occurred at one-twelfth of the retigabine (1-3 mg/kg) dose. Most importantly, CP86 treatment significantly reduced PTZ-induced mortality in mice, whereas retigabine was ineffective.[4] Altogether, these data suggest that CP86 is a potent, metabolically-stable, brain permeant, non-phototoxic retigabine derivative with high efficacy against chemically-induced seizures.

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