Identification and characterization of novel synthetic variabilin analogues targeting the glycine receptor Cl^- channels

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The glycine receptor (GlyR) chloride channel is a member of the pentameric Cys-loop ligand-gated ion channel family that mediates inhibitory neurotransmission in the spinal cord, retina and brainstem. Although α1-containing GlyRs are widely distributed, α3-containing GlyRs are expressed mainly in inhibitory synapses on spinal cord nociceptive neurons. The physiological consequences of differential α subunit distribution patterns are difficult to establish, as there are currently few pharmacological probes that can selectively modulate specific GlyR isoforms. Probes that specifically enhance α3 -containing GlyRs have emerged as potential targets for inflammatory pain. In 2010, we reported that variabilin analogues (isolated from marine sponge Ircinia variabilis) could be used as prototype for GlyR specific drug development (Balansa *et al.*, 2010). The difficulty in collection, and poor stability of natural isolates motivated us to synthesize variabilin metabolites, to ensure its sufficient supply for drug characterization and development. Hence, the aim of this study was to identify and characterize novel synthetic variabilin analogues that selectively modulate α1 or α3 GlyRs.

91 compounds were subjected to automated patch clamp based screening (NPC-16 Patchliner) on HEK cells that stably express either homomeric α1 GlyRs or α3 GlyRs cells. Around 18 hits were identified, including compounds that modulated both α1 GlyR or α3 and others that potentiated specifically either α1 or α3 GlyRs. CMB-C27-E11 potentiated α1 GlyR at lower doses (≤ 0.1μM) but inhibited at higher doses (≥30 μM). Among the compounds tested so far, compound CMB-C27-E11 (EC50 ~0.3μM) and CMB-C27-E2 (EC50 ~0.3μM) were found to be the strongest potentiators of α1 GlyR but compound CMB-C27-E2 (IC50 ~8.6 μM) was found to be a selective antagonist of α3 GlyRs, making it promising starting point for the development of lead compound for the treatment of temporal lobe epilepsy and other central nervous system disorders. Compounds CMB-C27-B10 (EC50 ~0.3μM), CMB-C27-B11 (EC50 ~0.3μM) and CMB-C27-B3 (EC50 ~1μM) potentiated current of both receptors but a stronger effect was found on α3 which making them useful pharmacological probes for inflammatory pain. We conclude that these synthetic variabilin analogues are potential candidates for drug developments targeting specific GlyRs isoforms.

Balansa W, Islam R, Fontaine F, Piggott AM, Zhang H, Webb TI, Gilbert DF, Lynch JW, Capon RJ. (2010) Ircinialactams: subunit selective glycine receptor modulators from Australian sponges of the family Ircinidae. *Bioorganic and Medicinal Chemistry* **18**: 2912-9.