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ABSTRACT

$GABA_A$ receptors are a class of receptors belonging to a superfamily of pentameric ligand-gated ion channels. Nineteen genes coding for different subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π and ρ1-3) have been identified in mammalians, and even though heteropentameric assembly leads to a huge subtype heterogeneity, it is believed that the most common receptors are composed of two α, two β, and a γ subunit. These receptors are targets of many clinically used compounds such as intravenous and volatile anesthetics or benzodiazepines, which modulate them via allosteric binding sites. Among the allosteric modulators identified so far, pyrazoloquinolinones (PQs) have been extensively studied in the last decades for their interesting pharmacological properties on $GABA_A$ receptors. These compounds are known to interact with at least three distinctive binding sites. Over the years, many modifications of the general PQ scaffold have been performed in order to accomplish compounds with better properties in terms of selectivity, potency and detectability. In this thesis we investigated modification of ring A, B and C of the PQ scaffold. On ring A, many modifications - both in terms of position of substituents and dimensions of the ring- have been explored; among them, the introduction of substituents in position R7 resulted in compounds with functional selectivity for α6β3γ2 receptors. In contrast, modifications on rings B and C are less investigated. Within this thesis, we further explored the impact of R7 on activity of PQ derivatives in α6β3γ2, synthesizing a library of compounds differently substituted in position 7. With the synthesized compounds, we observed two different patterns of allosteric modulation in recombinantly expressed α6β3γ2 receptors, namely monophasic and biphasic positive modulation. Furthermore, in order to gain more insight on the effect of changes at rings A and B, we used the pharmacophore of the PQ as a template to design a new scaffold, in which the size of ring B is reduced and the ring C is open. Herein we describe the synthesis and the biological properties of the resulting indole-derivatives on different receptor subtypes. The indole-derivatives share most of the pharmacophoric features with the PQs they were designed from. Contrary to what the pharmacophore overlap suggests, the indole-derivatives showed a different binding behavior compared to the PQs. To investigate the binding and mechanism of action of the new scaffold, we performed functional and mutational studies, as well as radioligand displacement assays. Computational docking studies complement the experimental findings in the context of the recently published cryo EM structure 6HUP.