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G-PROTEIN-COUPLED RECEPTORS

Targeting the hotspot of G-protein interactions

The interactions between Gβγ protein subunits and downstream effectors that transmit signals following ligand binding to G-protein-coupled receptors (GPCRs) are potentially attractive drug targets, but are also highly challenging because of the large surface area and flat topology of the interaction surfaces. However, in a recent paper in *Science*, Smrcka and colleagues have identified several compounds that differentially modulate interactions between βγ subunits of G-proteins and their effectors, demonstrating a novel approach for targeting GPCR signalling.

Although protein–protein interactions involve large interfaces, some studies have indicated the presence of 'hotspots' on the protein surfaces — small regions that are responsible for a large proportion of the affinity of interactions. Previous experiments by the Smrcka group involving the screening of phage-display libraries had identified peptides that bound such a hotspot on the surface of the $G\beta_1\gamma_2$ subunit. Intriguingly, these peptides could differentially affect the interaction of this subunit with various effectors.

The authors therefore set out to discover more drug-like small molecules that might target the same hotspot using structure-based virtual screening of ~2,000 compounds. The 85 highest-ranking compounds from this screen were then assessed for their capacity to bind to the $G\beta_1\gamma_2$ subunit by using an enzyme-linked immunosorbent assay based on competition with a known peptide binder.

Two compounds, M119 and M201, with affinities of 200–400nM were selected for further studies. Assays measuring their activity on Gβγ-mediated effectors revealed that although both compounds bound to Gβγ subunits and inhibited G-protein receptor kinase-2 binding to Gβγ, they differentially modulated Gβγ interactions with other effectors: M119 attenuated activation of phospholipase-Cβ2, phospholipase-Cβ3 and phosphatidylinositol 3-kinase (PI3K), whereas M201 did not affect phospholipase-Cβ2 activation, but potentiated activation of phospholipase-Cβ3 and PI3K. The two compounds also showed different capacities to modulate second messenger pathways in cellular systems: M119, but not M201, attenuated agonist-induced increases in intracellular calcium, whereas both compounds again inhibited G-protein receptor kinase-2 binding.

Finally, the authors examined the *in vivo* effect of M119 on the analgesic effects of morphine, which acts via μ-opioid GPCRs through a signalling pathway that is known to involve phospholipase-Cβ3. Co-administration of M119 with morphine resulted in an 11-fold increase in the analgesic potency of morphine, which is almost identical to the increse seen in phospholipase-Cβ3 knockout mice, further highlighting the specificity of M119.

Even though such compounds have yet to be tested in disease models, this research highlights that it is possible to identify molecules that selectively target the interaction hotspot between Gβγ subunits and their protein effectors, thereby increasing the possibility that such compounds might one day have potential as drugs for diseases such as heart failure for which Gβγ subunits have been identified as targets.

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ORIGINAL RESEARCH PAPER Bonacci, T. M. *et al.* Differential targeting of Gβγ-subunit signaling with small molecules *Science* **312**, 443–446 (2006)
FURTHER READING Arkins, M. R. & Wells, J. A. Small-molecule inhibitors of protein–protein interactions: progressing towards the dream. *Nature Rev. Drug Discov.* **3**, 301–317 (2004)