FUNCTIONAL AND STRUCTURAL UNDERSTANDING OF THE ANGIOTENSIN II RECEPTOR TYPE 1 (AT1R) BIASED SIGNALING

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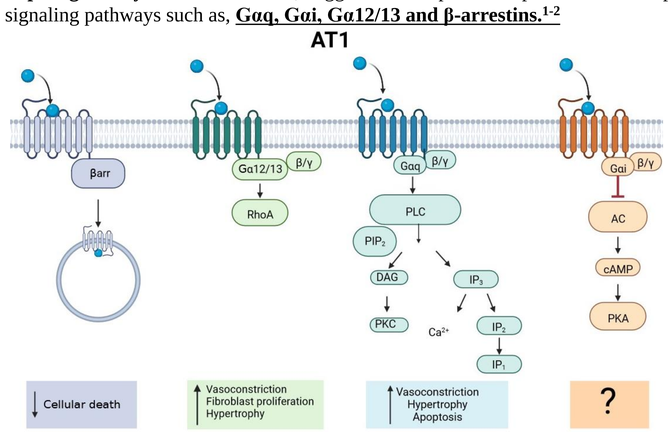
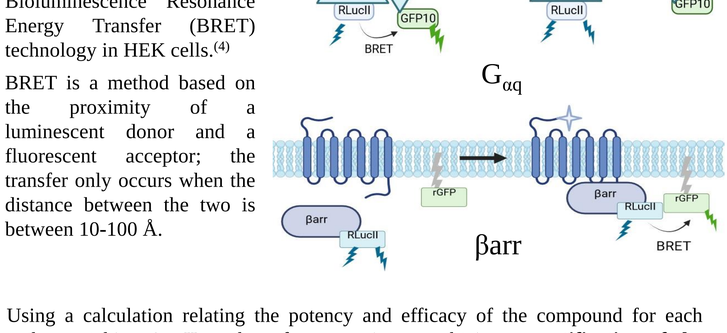
INTRODUCTION

AT1R belongs to the large family of G protein-coupled receptors (GPCRs) and is a central component of the renin-angiotensin system (RAS) with a major influence on **blood pressure regulation**. Its endogenous ligand angiotensin II (AngII), an octapeptide **Asp-Arg-Val-Tyr-Ile-His-Pro-Phe**, triggers both G protein-dependent and independent signaling pathways such as, **Gαq, Gαi, Gα12/13 and β-arrestins.**[1-2]



HYPOTHESIS

Selectively activating specific pathways would permit having positive effects (βarr) without the undesirable effects (Gαq). These types of ligands are called biased ligands. [3]

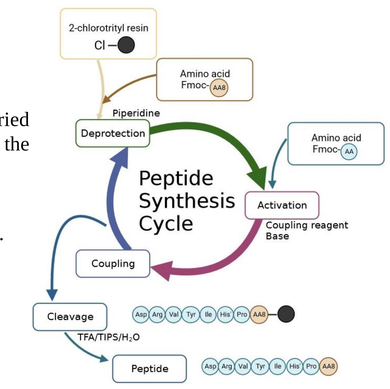


Objective: Understand the structure-signaling relationship of the Gq pathway.

METHODOLOGY

The peptide analogs' synthesis is carried out on solid phase (SPPS) following the Fmoc strategy.

Peptides are purified on PREP-HPLC.



The activation of the different pathways is studied using biosensors based on Bioluminescence Resonance Energy Transfer (BRET) technology in HEK cells.[4]

BRET is a method based on the proximity of a luminescent donor and a fluorescent acceptor; the transfer only occurs when the distance between the two is between 10-100 Å.

Using a calculation relating the potency and efficacy of the compound for each pathway, taking AngII as the reference point, we obtain a **quantification of the bias**.

Quantification of bias:

$$\Delta logR = \Delta log\left(\frac{\tau}{K_A}\right) = log\left(\frac{\tau}{K_A}\right)_{ligand} - log\left(\frac{\tau}{K_A}\right)_{AngII} \text{ or simplified as } \Delta log\left(\frac{Emax}{EC50}\right) = log\left(\frac{Emax}{EC50}\right)_{ligand} - log\left(\frac{Emax}{EC50}\right)_{AngII}$$

RESULTS

We replaced the Phe8 with a structural diversity of natural and unnatural amino acids. The Phe8 is known to play a crucial role in activating the Gq pathway without affecting βarr2 recruitment.(5)

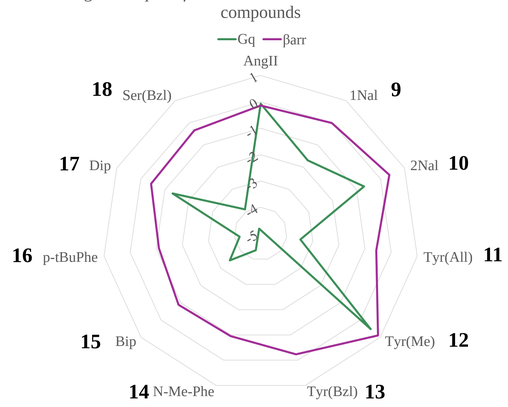
We aim to understand which ligand-receptor interactions are involved in the activation of the Gq pathway.

The replacement of Phe8 with lipophilic amino acids led to negatively Gαq-biased ligands able to recruit βarr to similar extent as AngII.

Polar or charged amino acids induce weak recruitment of βarr, owing to a potential lack of binding.

Spider plot Gq and βarr

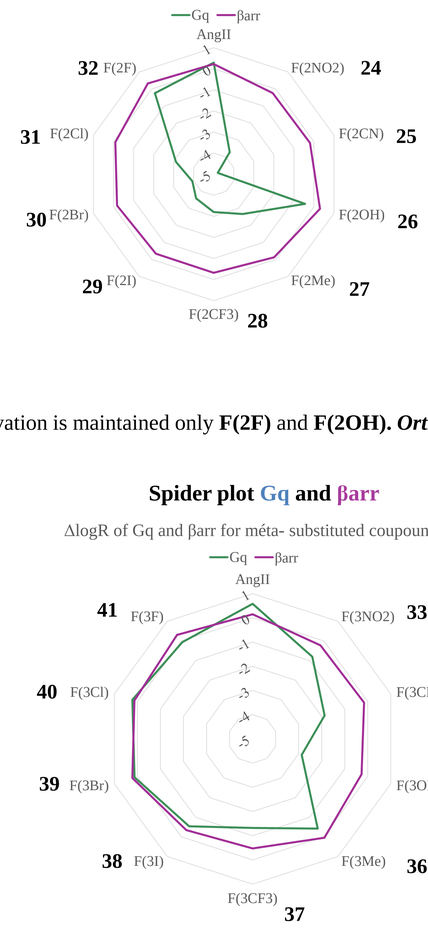
ΔlogR of Gq and βarr for aromatic amino acid substituted compounds



The size seems important, indeed amino acids with longer side chains lower proportionally the activation of Gaq. Moreover, expanding the width appears to result in a less pronounced impact on the activation of Gq ➔ Space in the interactions pocket

Spider plot Gq and βarr

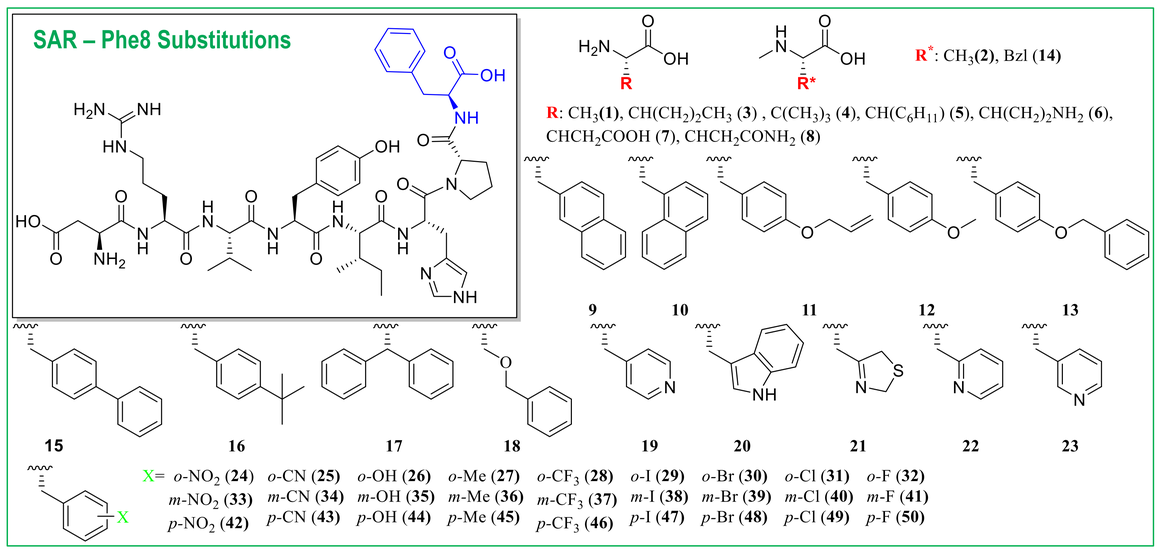
ΔlogR of Gq and βarr for ortho- substituted coupounds



Gq activation is maintained only **F(2F)** and **F(2OH)**. ***Ortho is highly sensitive.***

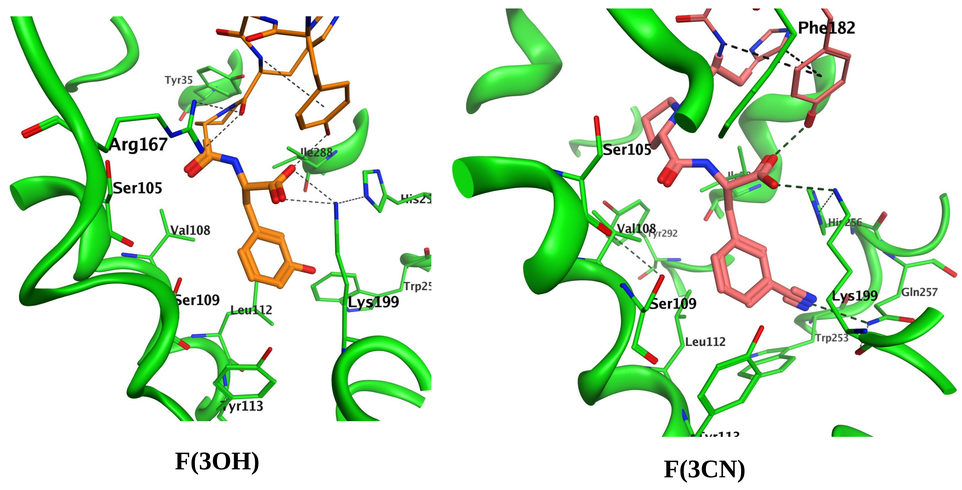
Spider plot Gq and βarr

ΔlogR of Gq and βarr for méta- substituted coupounds



Molecular Modeling

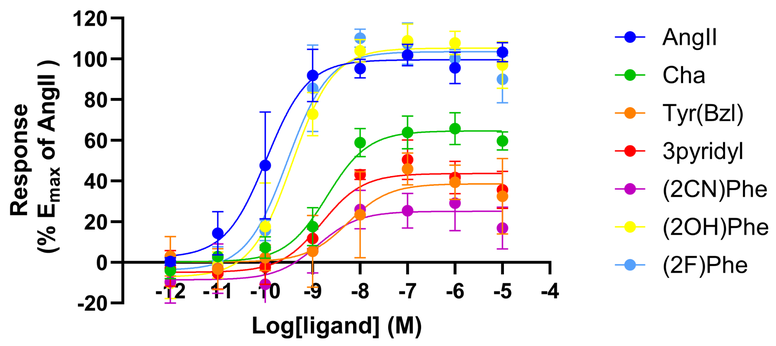
3D representation of 500 ns molecular dynamic simulations of AT1R (PDB 7F6G) with F(3CN) and F(3OH) Angiotensin II analogs



F(3OH) **F(3CN)**

Meta: ***H-bond donor group is detrimental***
Interaction with Gln257 could be implicated in Gq signaling

Protein Kinase C (PKC) downstream signaling



F(2CN), Tyr(Bzl), and 3pyridyl analogs of AngII exhibiting the lower activation of Gq protein also weakly trigger PKC signaling (Emax 20-40%).

CONCLUSION

We synthesized novel AngII analogs exhibiting various degrees of Gq protein and PKC activation, unraveling a chemical switch related to Gq signaling. Molecular modeling also provided new hypotheses about the interactions between ligand-AT1R restraining Gq activation.

PERSPECTIVES

- Biological tests: BRET in VSMCs, Blood Pressure Analysis in rats
- Crystallography: To understand which receptor conformation leads to the activation of Gq pathway

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