**TREPROSTINIL Prodrugs for Pulmonary Arterial Hypertension Evaluated in cAMP Profiling Studies in Live CHO-K1 Cells**


**INTRODUCTION**

TREPROSTINIL (TRE) is a prostacyclin analogue used to treat pulmonary arterial hypertension (PAH). It is primarily excreted as the metabolite 6-keto-prostaglandin F1α (6k-PGF1α), which generates cyclic adenosine monophosphate (cAMP) in cells that express prostacyclin (6k-PGF1α) receptors. The resulting increase in intracellular cAMP levels is thought to reduce muscle cell proliferation and enhance the relaxation of pulmonary arteries, leading to improved blood flow.

**METHODS**

**Treatment of CHO-K1 Cells**

CHO-K1 cells (ATCC CCL-61) were transiently co-transfected with pGloSensor-22F (Promega Corporation, Madison, WI) and EP2 plasmid (Promega Corporation, Madison, WI). cAMP is a cyclic nucleotide that plays a critical role in cellular signaling, particularly in the regulation of smooth muscle relaxation and blood vessel tone.

**Measurement of cAMP Levels and Kinetic Profiling**

cAMP levels were measured at predetermined time points to determine the kinetics of cAMP activation in response to TRE. The kinetics of cAMP production were analyzed using GraphPad Prism software to determine the half-life and maximum cAMP response.

**RESULTS**

**Optimisation of cAMP activation**

The data show that the cAMP activation profile in live CHO-K1 cells is concentration-dependent. The concentration of TRE was varied from 0 to 10 μM, and the cAMP activation was measured at different time points. The results indicate that the concentration of TRE significantly affects the cAMP activation profile.

**Effect of TRE on cAMP Activation**

The results show that the cAMP activation profile in live CHO-K1 cells is concentration-dependent. The concentration of TRE was varied from 0 to 10 μM, and the cAMP activation was measured at different time points. The results indicate that the concentration of TRE significantly affects the cAMP activation profile.

**Kinetic Profiling of cAMP**

The kinetic profiling of cAMP shows that the cAMP activation profile in live CHO-K1 cells is concentration-dependent. The concentration of TRE was varied from 0 to 10 μM, and the cAMP activation was measured at different time points. The results indicate that the concentration of TRE significantly affects the cAMP activation profile.

**CONCLUSIONS**

The study demonstrates that co-transfection with EP2 receptor plasmid successfully sensitized the signaling pathway of the commercially available cAMP assay for detecting cAMP activation in the presence of TRE. The results are consistent with previous studies, which have shown that the EP2 receptor plasmid increases the sensitivity of the commercially available cAMP assay for detecting cAMP activation.

**REFERENCE**


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