



Control of Inflammation in Pancreatic β -cells: Role of Arylpyrazole Compounds

Whitney L. Powell¹, Susan Burke¹, Carson Prevatte², Sara Daley³, Shawn Campagna², and J. Jason Collier¹

¹Department of Nutrition, University of Tennessee

²Department of Chemistry, University of Tennessee

³Department of Chemistry, Boston College



Abstract

Type 1 Diabetes Mellitus (T1DM) is an autoimmune disease characterized by the destruction of the insulin-producing pancreatic islet β -cells leading to elevations in blood glucose levels. Destruction of islet β -cells results from immune-cell infiltration into the islets and ensuing production of pro-inflammatory cytokines, such as interleukin-1 β (IL-1 β), from such cells. IL-1 β signals through receptors on the β -cell surface, which leads to the production of chemoattractant proteins called chemokines directly from the β -cells. This process produces a continuous inflammatory response that leads to β -cell death and dysfunction associated with the development of T1DM. Thus, novel therapeutic interventions that reduce or slow the progression of the inflammatory response within the pancreatic β -cells would be beneficial for treatment or cure of T1DM. One approach to reduce inflammation in a variety of human diseases is the administration of synthetic glucocorticoids. Glucocorticoids act through an intracellular receptor to elicit anti-inflammatory actions. However, current glucocorticoid receptor agonists impair β -cell function, which limits their clinical effectiveness. Thus, the current project investigated whether two non-steroidal arylpyrazole compounds were able to mimic the anti-inflammatory effects of glucocorticoids by suppressing known inflammatory responses in pancreatic β -cells. Using luciferase-based reporter assays as a measure of inflammatory gene activation revealed that Arylpyrazole 4 (AP4) significantly suppressed maximal IL-1 β response starting at 100nM whereas Arylpyrazole 5 (AP5) did not significantly suppress the IL-1 β response. When applied to a 3xGRE promoter, AP4 significantly activated the GRE at 100nM dosage whereas AP5 showed no activation of the GRE. The difference between these two compounds was an alcohol functional group present in the AP4 versus the ketone in AP5. We conclude that the difference in anti-inflammatory activity of AP4 and AP5 are due to distinct structure-function relationships with the ligand (AP) and glucocorticoid receptor. These preliminary findings will serve as the foundation for future investigation as to how the arylpyrazole scaffold can be modified to produce anti-inflammatory activities in pancreatic β -cells without suppressing insulin secretion.

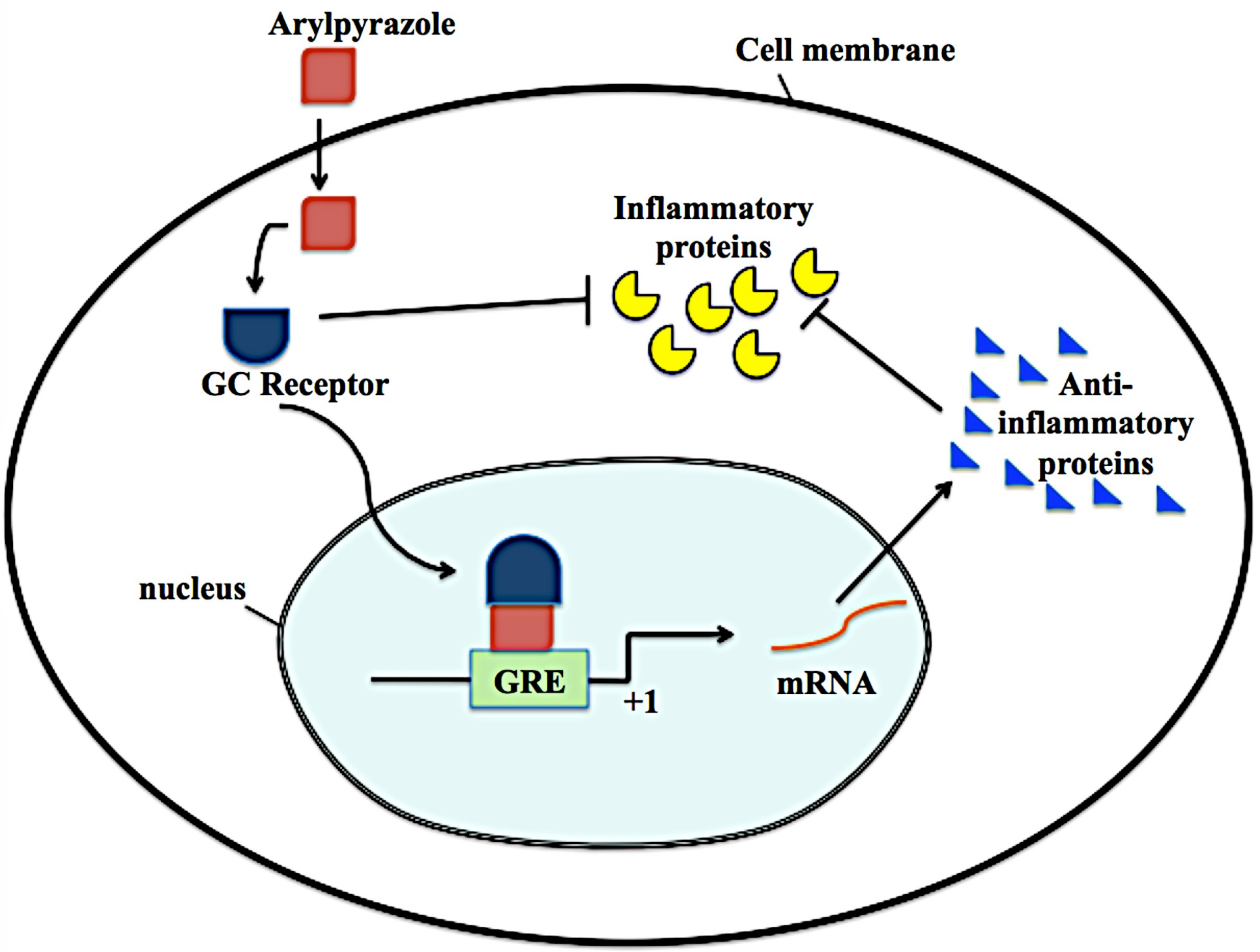


Figure 2. Schematic Representation of Arylpyrazole-mediated activation of anti-inflammatory proteins, depicting both genomic and non-genomic mechanisms of action. Genomic Mechanism. The arylpyrazole compound passes through the phospholipid bilayer membrane into the cytosol where it forms a dimer with the glucocorticoid (GC) receptor. This dimer passes into the nucleus where it binds to the glucocorticoid response element (GRE). This activates transcription of mRNA for the biosynthesis of anti-inflammatory proteins. Non-genomic Mechanism. The arylpyrazole-GC receptor dimer can directly inhibit the activity of the inflammatory proteins within the cytosol.

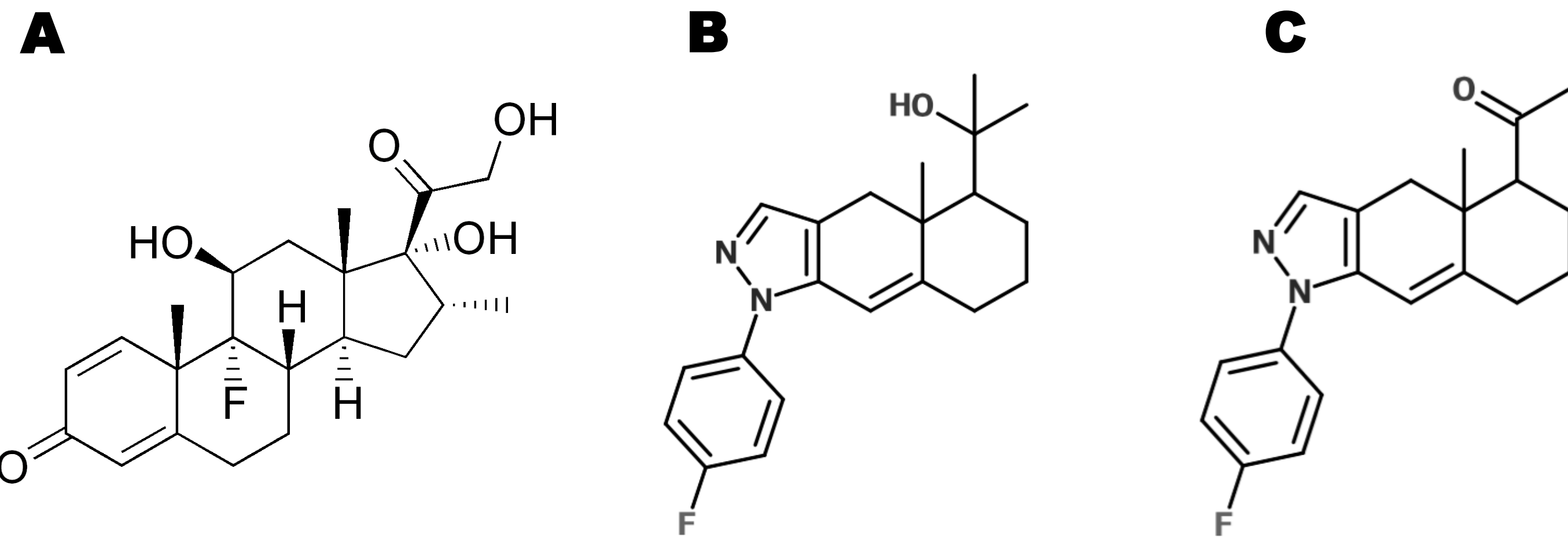


Figure 4. Chemical Structures of Dexamethasone, Arylpyrazole 4, and Arylpyrazole 5 contribute to different downstream anti-inflammatory activity. *A.* Dexamethasone, a synthetic glucocorticoid, possesses many reactive alcohol functional groups, contributing to its ability to suppress IL-1 β signaling and activation of the GRE. *B.* Arylpyrazole 4 possesses a reactive alcohol group, which mediates its affinity as a ligand for intracellular glucocorticoid receptors. *C.* The ketone functional group in Arylpyrazole 5 contributed to its inability to significantly suppress IL-1 β signaling.

Summary and Conclusions

- Arylpyrazole 4 inhibits IL-1 β -mediated CCL2 transcription by $\geq 50\%$ in the 832/13 cell line.
- Arylpyrazole 4 activated transcription from a synthetic GRE containing a luciferase promoter.
- Arylpyrazole 5 did not significantly inhibit pro-inflammatory cytokine IL-1 β activity compared to the control.
- Arylpyrazole 5 showed no activation of the GRE promoter compared to the control.

Future Research

Understanding the specific role of cytokines as a cellular signal in both the innate and adaptive immune responses would undoubtedly aid in unveiling the components of the inappropriate immune response in T1DM. Thus, future research endeavors should investigate how cytokines regulate the interplay of various types of immune cells during the development of autoimmune diseases. Another area of study involves investigating the divergent mechanisms by which the intracellular glucocorticoid receptor functions to inhibit and activate genetic expression as this would aid in the development of novel drugs utilized to treat individuals with T1DM.

References

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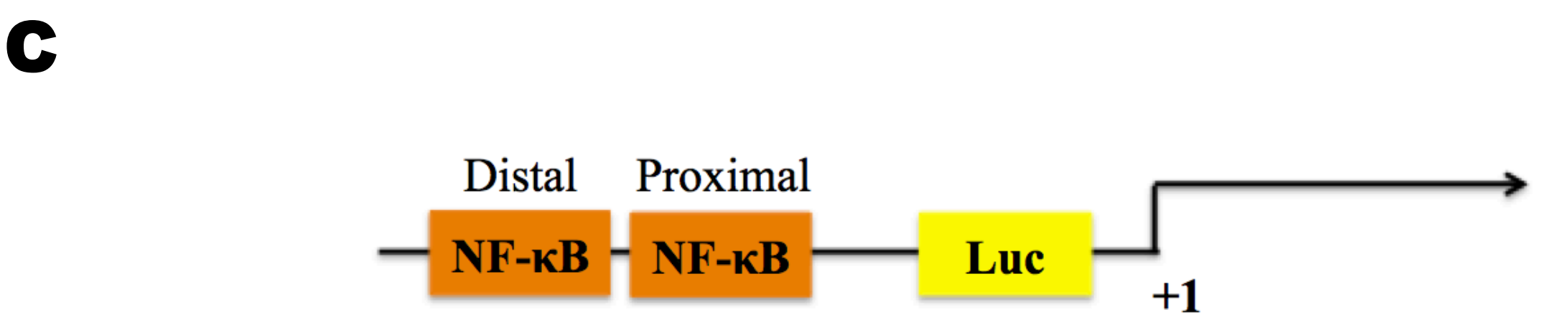
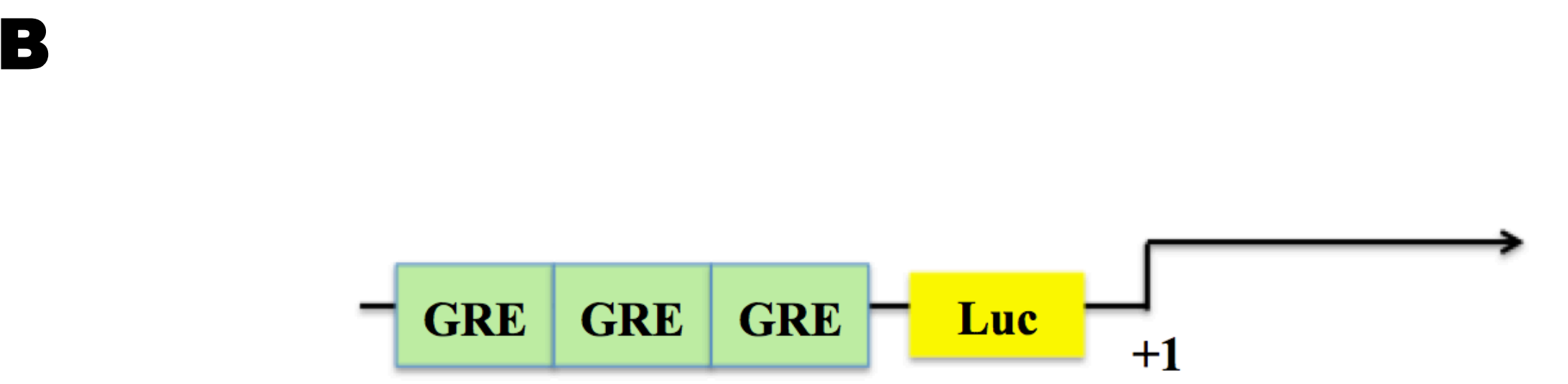
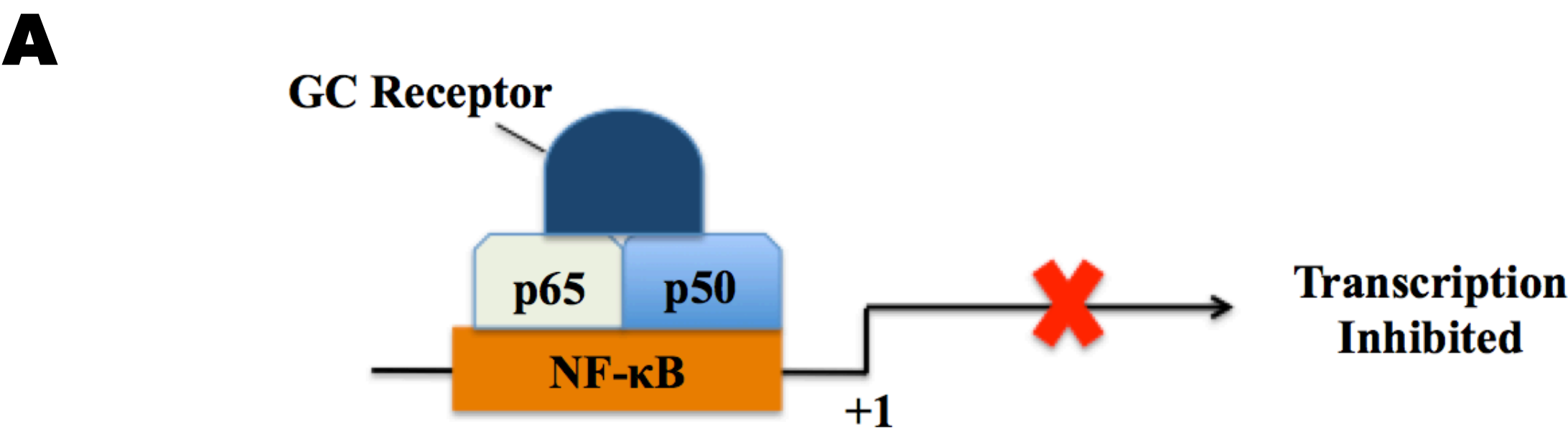


Figure 1. Schematic Representation of Genomic Sites Targeted by Intracellular Glucocorticoid Receptors.

A. Recruitment of Glucocorticoid receptors to the NF- κ B promoter site can inhibit the transcription of pro-inflammatory agents. *B.* A synthetic promoter consisting of three back-to-back GRE sites and a luciferase gene downstream. *C.* A promoter consisting of two NF- κ B sites and a luciferase gene downstream.

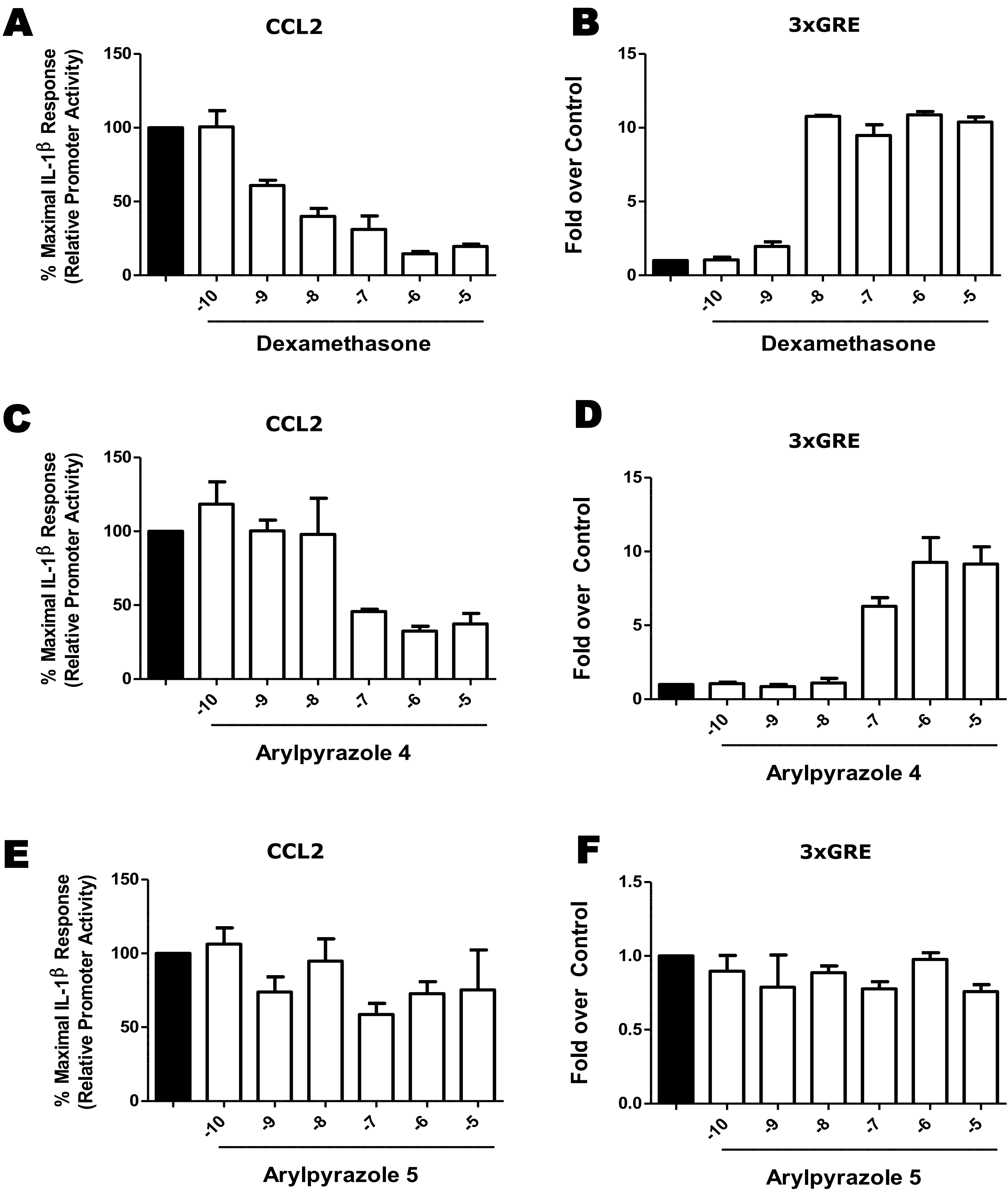


Figure 3. Arylpyrazole 4 inhibits cytokine IL-1 β activity and activates GRE while Arylpyrazole 5 neither inhibits cytokine IL-1 β activity nor activates GRE. *A.* 832/13 cells were transfected with a plasmid containing the CCL2 (Chemokine ligand 2) promoter upstream of a luciferase gene. Cells were treated with IL-1 β in the absence or presence of increasing doses of Dexamethasone (Dex) for 4 hours. The control consisted of cells only treated with IL-1 β and was set at 100% maximal IL-1 β response. *B.* 832/13 cells were transfected with a plasmid containing a 3xGRE (glucocorticoid response element) promoter upstream of a luciferase gene. Cells were treated with increasing doses of Dex for 4 hours. The control consisted of cells only treated with dimethyl sulfoxide (DSMO). *C.* 832/13 cells were transfected with a plasmid containing the CCL2 promoter driving expression of a luciferase gene and were treated with IL-1 β plus increasing doses of Arylpyrazole 4 for 4 hours. The control consisted of cells only treated with IL-1 β and was set at 100% maximal IL-1 β response. *D.* 832/13 cells were transfected with a plasmid containing 3x GRE promoter upstream of a luciferase gene and treated with increasing doses of Arylpyrazole 4 for 4 hours. The control consisted of cells only treated with DSMO. *E.* 832/13 cells were transfected with a plasmid containing the CCL2 promoter upstream of a luciferase gene and treated with IL-1 β plus increasing doses of Arylpyrazole 5 for 4 hours. The control consisted of cells only treated with IL-1 β and was set at 100% maximal IL-1 β response. *F.* 832/13 cells were transfected with a plasmid containing a 3xGRE promoter upstream of a luciferase gene and treated with increasing doses of Arylpyrazole 5 for 4 hours. The control consisted of cells only treated with DSMO.