# Identification of Toll-Like Receptor 2 (TLR2) Antagonists Using Computer-Aided Drug Design

## Summary

Toll-like receptors (TLR) are innate immune molecules involved in the detection of microbial and self-ligands released from damaged cells. At their C-termini, toll/interleukin-1 receptor (TIR) domains dimerize upon ligand binding, initiating a signaling cascade that results in the upregulation of inflammatory cytokines. Excessive signaling can lead to a cytokine storm, a potentially fatal immune reaction. Dysregulated TLR2 signaling has been explicitly linked to conditions involving the production of proinflammatory cytokines, such as sepsis, atherosclerosis, tumor metastasis, and ischemia/reperfusion injury. Based on the published crystal structure of the TIR domain of TLR2, UMB researchers used computer-aided drug design (CADD) to identify small molecule inhibitors that may disrupt TLR2 signaling. This *in silico* screening identified 169 small molecule TLR2 inhibitors that potentially disrupt TIR-TIR interactions. Some of these compounds demonstrated *in vitro* and *in vivo* selective TLR2 inhibition and the ability to inhibit TLR2-induced inflammation.

### Key Investigator
Alexander D. MacKerell
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### Field
Drug discovery

### Technology
Small molecule TLR2 inhibitors

### Advantages
Small molecule inhibitors of TIR-specific TLR signaling

Potential to identify additional structurally-related compounds

Therapeutic potential in conditions involving TLR2 signaling such as sepsis and inflammation

### Status
Available for licensing
Available for sponsored research

### Patent Status
WO 2016/164414 A1

### UMB Docket Reference
SV-2010-075

### External Reference
Mistry P et al. (2015)
*PNAS*. 112(17):5455-60.

## Market

As modulators of innate immunity, the scientific literature supports the use of TLR2 inhibitors as potential therapeutics for the prevention and treatment of autoimmune and inflammatory diseases. Despite the promise of TLR2 antagonists, few compounds targeting this complex have been developed. Currently, there is only one TLR2 signaling inhibitor in clinical development. This TLR2 inhibitor is a humanized monoclonal antibody for patients with myelodysplastic syndrome and for the prevention of delayed graft function following renal transplantation.

## Technology

Using CADD, approximately 150 molecules and 20 FDA-approved drugs were identified as candidate inhibitors. Drug candidates were screened for their ability to block TLR2 ligand-mediated signaling leading in a humanized cell line, with some compounds further screened for specificity in murine and human macrophages using non-TLR2 ligands. Several potential TLR2-specific antagonists have been identified, as well as compounds with broader inhibitory capacity. Based on the structure of these active compounds, additional structurally related compounds can be identified.
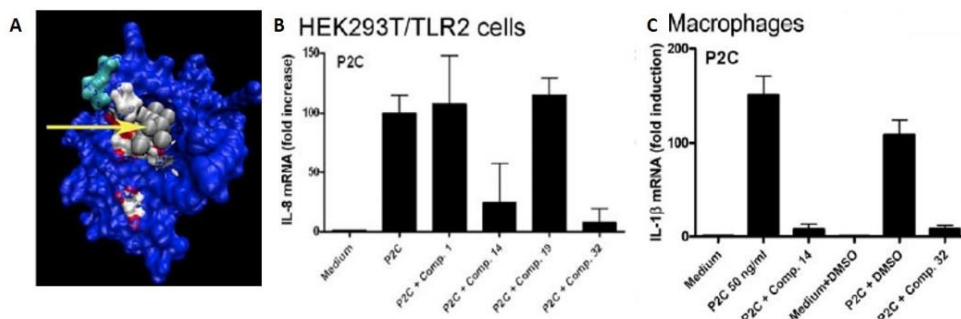


(A) 3D representation of TLR2 TIR domain. (B) Inhibition of P2C-induced IL-8 mRNA (measured by qrtPCR) in HEK293T cells stably expressing human TLR2 by compounds #14 and #32, but not by compounds #1 and #19 (selected); (C) Inhibition of P2C- and P3C-induced IL-1β mRNA in thioglycollate-elicited, primary, murine peritoneal macrophages by compounds #14 and #32. N>3 independent experiments. Cytokine gene expression normalized to GAPDH.

## Technology Status

Drug candidates were screened for TLR2 inhibitory activity in a human cell line (HEK293T) that expresses TLR2. Ideal candidates were identified by their ability to block TLR2 ligand-mediated signaling leading to IL-8 mRNA expression. These lead candidates were further tested for TLR2 specificity in murine and human macrophages. The ability of some compounds to inhibit TLR2/1-induced proinflammatory cytokines was also tested *in vivo* in mice.

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