Discovery, Synthesis and Biological Evaluation of Small-Molecule Inhibitors of Toll-like Receptor Signaling Pathways

by

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The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.

Abstract

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Thesis directed by Professor Hang Hubert Yin

Toll-like receptors (TLRs) are essential to the innate immune system. These receptors help drive inflammatory responses, host defense, and adaptive immune responses upon detection of invading microorganisms. Small molecules capable of targeting TLR signaling are of considerable interest as potential therapeutic agents for the treatment of human inflammatory diseases and cancers that are caused by dysregulation of TLR signaling. The goal of this doctoral dissertation research is to identify small-molecule modulators of TLR signaling via high-throughput screening (HTS) approach followed by structure-activity relationship (SAR) studies.

Chapter 1 is an overview of the current knowledge of TLRs, including their structures and functions, signaling pathways, as well as their roles in inflammatory diseases and cancer. The current status of TLR agonists and antagonists in clinical studies is also summarized.

Chapter 2 focuses on the development of a new family of TLR4 signaling inhibitors, identified from a cell-based screening. A series of arylidene malonate analogs were synthesized and assayed in murine macrophages for their inhibitory activity against LPS-induced nitric oxide (NO) production. The lead compound, **1** (**NCI126224**), was found to suppress LPS-induced production of nuclear factor-kappaB (NF-κB), tumor necrosis factor (TNF-α), interleukin-1β (IL-1β), and nitric oxide (NO) in the nanomolar to low micromolar range.

Chapter 3 describes a high-throughput compound library screening to identify novel TLR8 signaling antagonists. The Maybridge Hitfinder library of 14,400 compounds was screened using SEAP reporter cells expressing TLR8. The screen yielded thirteen novel TLR8 signaling specific inhibitors. Structure-activity relationship investigation and biological evaluation were mainly focused on one hit **40-D4** with a pyrazolo[1,5-*a*]pyrimidine scaffold. Two lead compounds, **8m** and **4m**, were identified with nanomolar potencies inhibiting TLR8 signaling. Further biological evaluation indicates that **8m** specifically inhibits TLR8 signaling without affecting other TLRs. **8m** also suppresses TLR8 induced proinflammatory cytokine and cytokine mRNA levels. These finding suggests that these compounds may have therapeutic applications in the treatment of TLR8-related inflammatory diseases.

Chapter 4 described a study which utilized both *in silico* and cell-based screening to identify agonists and antagonists of TLR5 signaling. One potential TLR5 signaling inhibitor was identified from cell-based HTS.

Chapter 5 summarizes the major findings of this dissertation research, illustrates the potential of the identified small-molecule suppressors, and explores future research directions for understanding the mechanism of these TLR inhibitors.