Small Molecule perturbation of the TLR4/MD-2 Interaction

by

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Small Molecule perturbation of the TLR4/MD-2 Interaction

Hubert Yin

Tarek Sammakia

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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 Hubert Yin

Opioid-induced glial cell activation has accumulated great interest over the past several years. Recent evidence suggests that glia are activated by opioids and that the activation of glial cells is linked to suppression in analgesia, leading to the development of opioid tolerance and dependence. Current study shows that opioid-induced glial activation is regulated by Toll-Like receptor-4(TLR4), a membrane spanning receptor that functions in complex with Myeloid differentiation factor-2 (MD-2). Interestingly TLR4 receptors are solely expressed by glia within the central nervous system (CNS), suggesting development of small molecules to improve opioid-based pain management therapies through the inhibition of the TLR4/MD-2 interaction in glial cells within the CNS. Initially the high-resolution crystal structure of the TLR4 extracellular domain (2z62) was screened against an ENAMINE collection (a library of over 700,000 compounds) in an ADMET *in silico* screen. This led to the identification of a handful of potential lead compounds. Initially every compound was tested through the use of a secreted alkaline phosphatase (SEAP) assay to measure TLR4 activation inhibition. Upon screening the hits, two leads were indentified for further testing, compounds 2126 and 4019. Both compounds were subject to further testing using a variety of cell and biophysical assays, including RAW nitric oxide production, Co-Immunoprecipitation, TLR-specificity assays, and Glial IL-1β production via enzyme linked immunosorbent assays done in conjunction with animal

testing performed by Dr. Linda Watkins. These studies have allowed us to shed light on the action of our small molecules leads and their analogs both *in vitro* and *in vivo.* Although further study is needed, these small molecules provide an exciting avenue for developing drug-like therapeutics to enhance opioid analgesia while minimizing opioid dependence and tolerance.