Identification and Characterization of Small Molecule Nucleotide Binding and Oligomerization Domain 2 (NOD2) Receptor Agonists

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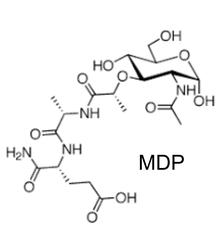
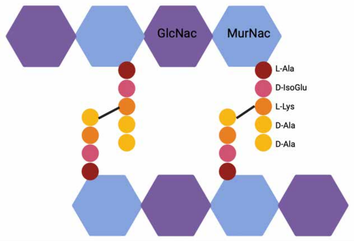
https://doi.org/10.17952/37EPS.2024.P2202

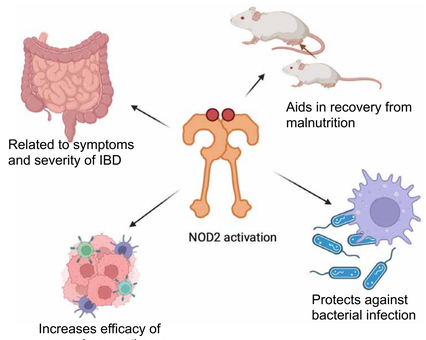
Background

The nucleotide binding and oligomerization domain protein 2 (NOD2) is an innate immune receptor that detects fragments a portion of the bacterial cell wall, known as peptidoglycan (PG).[1] PG is a polymer of crosslinked units of N-acetyl glucosamine (GlcNac) and N-acetyl muramic acid (MurNac), with a short peptide chain attached to MurNac. The peptide chain typically has the sequence L-Ala-D-isoGlu-L-Lys-D-Ala-D-Ala, with some organisms having a mesodiaminopimelic acid instead.[1]



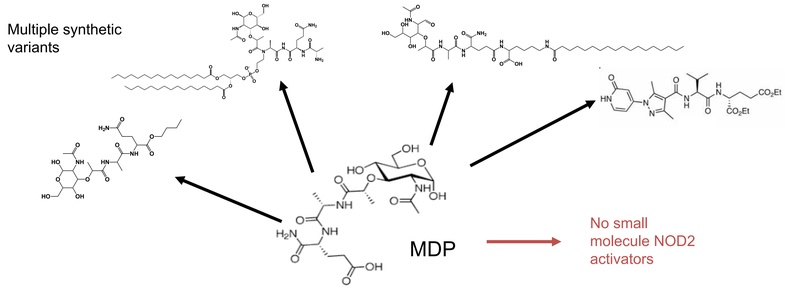


NOD2 activation has been shown to have many beneficial effects, such as protecting against bacterial infections,[2] aiding in recovery from malnutrition,[3] reducing symptoms of irritable bowel diseases,[4] and increasing the efficacy of cancer immunotherapy.[5] The minimal active fragment of peptidoglycan, muramyl dipeptide (MDP), composed of MurNac and the first two amino acids in the stem peptide, has been shown to cause these beneficial effects.



However, MDP is pyrogenic and difficult to use as a treatment in clinical settings. Other avenues to increase NOD2 activation, such as probiotic bacteria, are difficult to control in the body and hard to elucidate the exact effects from treatment. Many synthetic variations on MDP are currently available and used, but no unrelated small molecule activators are options for widespread use.

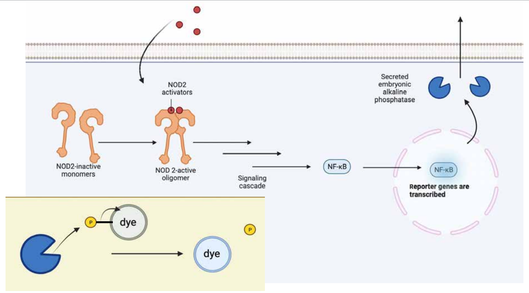
Project Aims



DISCOVER SMALL MOLECULE ACTIVATORS OF NOD2 THAT POTENTIATE THE SAME EFFECTS AS MDP

Methods and Results

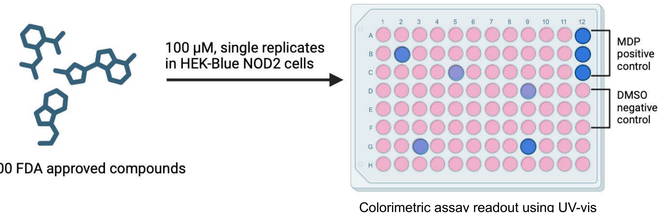
Method Overview: HEK-Blue™ NOD2 cells



- HEK cells that express a reported gene downstream of NF-kB
- NOD2 activation causes expression of reporter genes
- Dye compound in media is dephosphorylated, causing color change
- Read results at 655 nm on UV-vis spectrophotometer

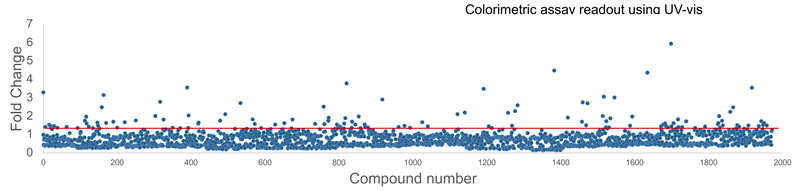
Discovery and Characterization of a Small Molecule NOD2 Activator

Screen of FDA Approved Compound Library



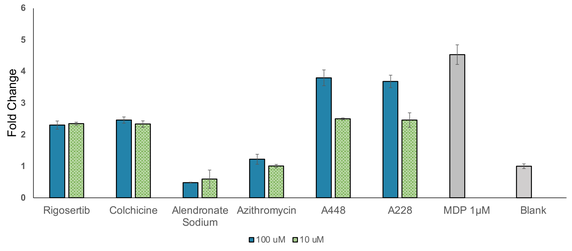
~2000 FDA approved compounds

Colorimetric assay readout using UV-vis



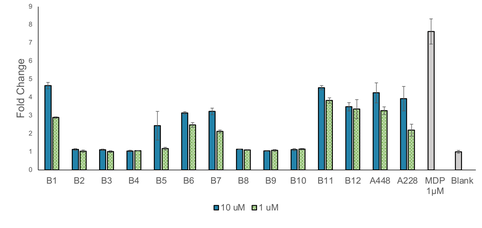
At a cutoff of fold change 1.5, several NOD2 agonists were identified

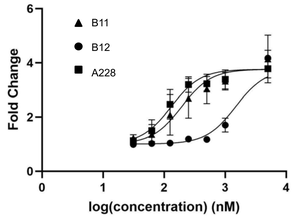
Further Analysis of Screen Hits



Strongest hits 14-48 and 20-28 share major structural similarities.

Expansion of Structurally Similar Screening Hits



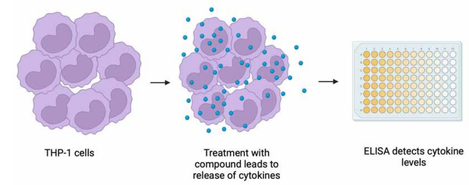


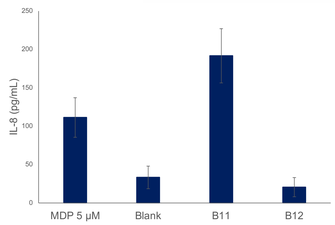
Several compounds with a similar core chemical structure activate NOD2. Two especially potent agonists (B11, B12) were identified

Methods and Results

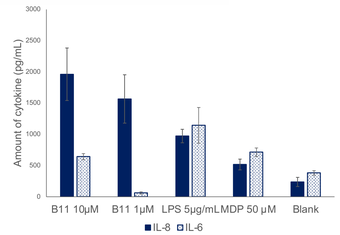
Inhibition and Cytokine Release Small Molecule NOD2 Activator

Cytokine Release by Screen Hits in THP-1 cells



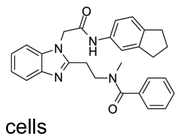


B11 induces cytokine release more strongly than B12

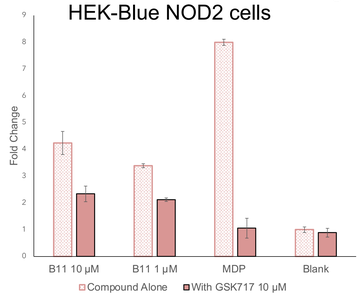


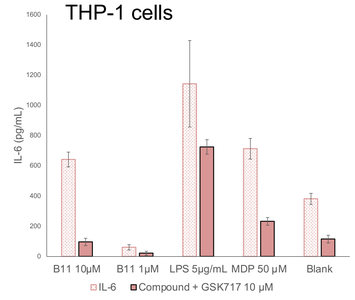
B11 induces IL-8 and IL-6 production

Inhibition by Specific NOD2 Inhibitor GSK717



GSK-717





GSK717 inhibits activation of NOD2 by B11 in HEK-Blue NOD2 cells, and blocks cytokine release in THP-1 cells, indicating that B11 works through NOD2

Ongoing Work

- ❑ Structural activity relationship studies with hit compounds to improve activation
- ❑ Cytokine panel using bone marrow derived macrophages
- ❑ Photocrosslinking assays to better demonstrate specific interaction with NOD2

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