Identification of GSK-3 Beta Inhibitors from Natural Products

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INTRODUCTION:

- Currently, there are very few drugs available to treat Alzheimer's disease and these drugs do not reverse or stop the disease from progressing. Many of them work by inhibiting acetylcholinesterase, which only moderately helps maintain cholinergic nerve function but does not stop neuronal death.
- GSK-3β is an enzyme that phosphorylates tau proteins in the brain. If tau proteins become hyperphosphorylated, they break apart and aggregate, forming the neurotoxic tau tangles that lead to Alzheimer's Disease. In theory, inhibition of GSK-3β will prevent neurotoxic tangles that cause cholinergic neuron death.
- Our purpose was to identify new GSK-3β inhibitors from plant sources. Potent natural inhibitors could make it possible to take dietary supplements or eat certain foods to decrease the risk of developing Alzheimer's Disease.

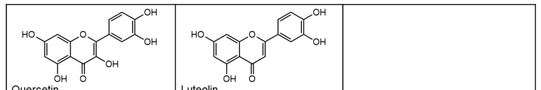
METHODS

1. Ligand Screening
- Compounds known to inhibit GSK-3β were identified using PubChem.
- A database of herbal compounds was screened using computer modeling to identify natural products structurally like known GSK-3β inhibitors.

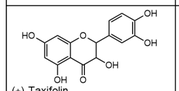
2. Compound Testing
- Identified compounds were purchased and dissolved in 10% DMSO.
- Compounds were tested at a concentration of 100 µM in a 96 well plate with active GSK-3β enzyme and ATP.
- Negative control wells (no enzyme) and positive control wells (no inhibitors) were included on the plate, along with wells containing known GSK-3β inhibitors like quercetin and luteolin.
- Kinase-Glo® Plus was used to measure enzyme activity in each well. This reagent luminesces in proportion to the amount of ATP in the well.
  - More enzyme inhibition = more unused ATP = more luminescence

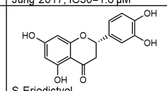
RESULTS

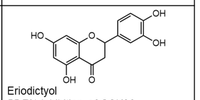
- Compounds from the flavanone class of flavonoids, found primarily in citrus, inhibited GSK-3β with potencies that varied based on their stereochemistry and arrangement of alcohol groups.
- S-Eriodictyol rivaled some known flavonoid inhibitors in its potency.

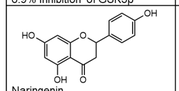

Quercetin
Flavone Positive Control
82.7% Inhibition of GSK3β

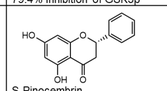
Luteolin
Flavone Positive Control
98.3% Inhibition of GSK3β
Jung 2017, IC50=1.6 µM

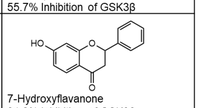

(±)-Taxifolin
6.9% Inhibition of GSK3β

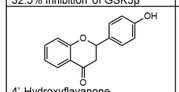

S-Eriodictyol
79.4% Inhibition of GSK3β

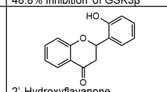

Eriodictyol
55.7% Inhibition of GSK3β

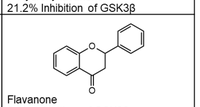

Naringenin
32.5% Inhibition of GSK3β


S-Pinocembrin
48.8% Inhibition of GSK3β


7-Hydroxyflavanone
21.2% Inhibition of GSK3β


4'-Hydroxyflavanone
0.4% Inhibition of GSK3β


2'-Hydroxyflavanone
0.2% Inhibition of GSK3β


Flavanone
0.2% Inhibition of GSK3β

BOTTOM LINE

The structural basis for GSK-3β inhibition by a series of citrus flavanones was identified.

However, currently known flavonoids from foods are unlikely to inhibit GSK-3β in vivo because of their inadequate potency and rapid metabolism.

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Some Citrus Flavonoids Inhibit the Alzheimer's Target GSK-3 Beta at High Concentrations

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