ISOLATION AND CHARACTERIZATION OF BIOACTIVE PSEUDOGUAIANOLIDES FROM *HELENIUM FLEXUOSUM* SEEDS

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In 2010, we have initiated a systematic screening of plant seeds for their antibacterial potential.

Natural product based drug discovery played a crucial role in the development of modern medicine\textsuperscript{1}.

Almost 70\% of antibacterial drugs are either natural products or are derived from natural products\textsuperscript{2}.

\begin{itemize}
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Why screen for new antibacterials?

• Because…

• Some pathogens stopped accepting VISA.

• VISA stands for vancomycin intermediate staphylococcus aureus.

• A veritable list of acronyms (MRSA, VISA, VRSA)

• The list is so long it ESKAPEs my memory.
Discovery gap

- The early excitement in antibiotic drug development tapered off by the 60s\(^3\).

A few structural classes

- Quinolones
- Macrolides
- Tetracyclines
Our screening of plant seed extracts led to the prioritization of two plants: *Helenium flexuosum* and *Helenium autumnale* (Family: Asteraceae).

Chemical examination of *H. autumnale* resulted in the characterization of two known flavonoids.
Helenium flexuosum

A species of annual herb in the daisy family known by the common name purple sneezeweed. It is native to the eastern half of North America.
Method

• *H. flexuosum* seeds (454 g) were powdered and extracted repeatedly with methanol and the combined methanol was evaporated on a rotavapor.

• The crude extract showed activity against *S. aurues* in the disc diffusion assay.
Kupchan partition

- The residue from the methanol extract was subjected to Kupchan partition. The dichloromethane fraction inhibited *S. aureus* moderately in disc diffusion assay.
Chromatography

Larger fractions were separated on glass columns using gravity to drive the solvent flow.

As fractions get smaller, Biotage system was used.
Chromatography

• Repeated chromatography of the dichloromethane fraction on normal and reverse phase columns using a Biotage flash chromatography system resulted in a number of fractions.
Fractionation Chart

Heliumium Flexuosa Seed

HF F004

HF F003

HF F002

HF F001
Purification of selected fractions

- F026 was initially purified using DCM:Methanol (93:7) on a burette column.

- F035 from F026 was further purified using DCM:Methanol (98:2). Purity of the fractions was checked by TLC using hexane:acetone (1:1).

- The final purification was done using toluene:acetone (7:3) to obtain 4 mg (0.0001 % dry weight) of F041 (isohelenol). UV light was used to visualize compounds on TLC followed by heating the TLC plate after dipping it in Methanol:H₂SO₄ (9:1).
Fractions visualized under UV light
TLC of isohelenol and related fractions
Results

• Thin Layer Chromatography and $^1$H NMR analysis of these fractions suggested the presence of closely related compounds.

• Isohelenol, a sesquiterpene lactone, was characterized using 1D and 2D NMR.

• It was previously reported from *Helenium microcephalum* and was reported to have *in vivo* activity against mouse P388 lymphocytic leukemia.
$^{13}$C NMR spectrum of Isohelenol
\(^1\text{H NMR spectrum of Isohelenol (C}_{15}\text{H}_{18}\text{O}_5)}\)
$^1\text{H}-^1\text{H}$ COSY spectrum of Isohelenol
HMQC spectrum of Isohelenol
Other compounds in the process of characterization

F051

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\begin{align*}
\text{R} &= \text{C(O)-CH(\text{CH}_3)-\text{CH}_2-\text{CH}_3}
\end{align*}
\]
Sesquiterpene lactones

- Sesquiterpene lactones (SLs) are plant-derived compounds often used in traditional medicine against inflammation and cancer.

- A few sesquiterpene lactone derived drugs from artemisinin, thapsigargin and parthenolide are currently in clinical trials.
Importance of SLs

• The chemical properties of SLs comprise alkylation center reactivity, lipophilicity, and molecular geometry and electronic features\(^4\).

• These drugs are selective toward tumor and cancer stem cells by targeting specific signaling pathways, which make them lead compounds in cancer clinical trials.

Future work

- Complete the characterization of SLs from H. flexuosum
- Send them for testing to NCI.
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