Supporting Information

Inhibition of HIF-1α through Suppression of NF-κB Activation by Compounds Isolated from Senecio graveolens

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Abstract

One of the characteristics of cancer is that the lack of oxygen in the cancer cells triggers changes in their gene expression. This hypoxia activates hypoxia-inducible factor 1-alpha and this in turn sets in motion the whole family of important angiogenic genes for the tumour. Hypoxia-inducible factor 1-alpha therefore increases the density and vascular permeability within the tumours, facilitating their rapid growth and, later, the metastasis. *Senecio graveolens* is a South American medicinal plant commonly used for mountain sickness (lack of adaptation of the organism to hypoxia). Additionally, pharmacological studies showed that its alcoholic extracts have cytotoxic properties.

This research aimed to perform a guided phytochemical study of *S. graveolens* to identify compounds capable of inhibiting hypoxia-inducible factor 1-alpha through suppression of nuclear factor kappa-light-chain-enhancer of activated B cell activation. The isolation led to the characterisation of phanurane (1), damsine (2), and scoparone (3), first reported in the *S. graveolens* species.

Phanurane (1) showed inhibitory activity of hypoxia-inducible factor 1-alpha on the cancer cell lines U-373 MG (IC\(_{50}\) = 20.66 ± 0.04 μM), A549 (IC\(_{50}\) = 25.80 ± 0.04 μM), Hep G2 (IC\(_{50}\) = 29.21 ± 0.03 μM), and Caco-2 (IC\(_{50}\) = 38.58 ± 0.02 μM). Damsine (2) hypoxia-inducible factor 1-alpha displayed inhibitory activity of hypoxia-inducible factor 1-alpha on the cancer cell lines U-373 MG (IC\(_{50}\) = 2.29 ± 0.07 μM), A549 (IC\(_{50}\) = 4.13 ± 0.04 μM), Hep G2 (IC\(_{50}\) = 6.40 ± 0.03 μM), and Caco-2 (IC\(_{50}\) = 9.80 ± 0.04 μM). Finally, scoparone (3) displayed inhibitory activity of hypoxia-inducible factor 1-alpha on the cancer cell lines U-373 MG (IC\(_{50}\) = 15.22 ± 0.01 μM), A549 (IC\(_{50}\) = 17.47 ± 0.02 μM), Hep G2 (IC\(_{50}\) = 18.26 ± 0.06 μM), and Caco-2 (IC\(_{50}\) = 19.75 ± 0.04 μM).

In addition, phanurane (1) displayed inhibitory activity over nuclear factor kappa-light-chain-enhancer of activated B cells on cancer cell lines U-373 MG (IC\(_{50}\) = 7.13 ± 0.03 μM), A549 (IC\(_{50}\) = 8.64 ± 0.03 μM), Hep G2 (IC\(_{50}\) = 8.87 ± 0.04 μM), and Caco-2 (IC\(_{50}\) = 15.11 ± 0.01 μM). Likewise,
damsine (2) showed inhibitory activity over nuclear factor kappa-light-chain-enhancer of activated B cells on cancer cell lines U-373 MG (IC$_{50}$ = 2.28 ± 0.01 μM), A549 (IC$_{50}$ = 3.79 ± 0.02 μM), Hep G2 (IC$_{50}$ = 3.98 ± 0.05 μM), and Caco-2 (IC$_{50}$ = 6.41 ± 0.02 μM). Lastly, scoparone (3) displayed inhibitory activity of nuclear factor kappa-light-chain-enhancer of activated B cells on cancer cell lines U-373 MG (IC$_{50}$ = 3.62 ± 0.06 μM), A549 (IC$_{50}$ = 4.48 ± 0.03 μM), Hep G2 (IC$_{50}$ = 5.25 ± 0.01 μM), and Caco-2 (IC$_{50}$ = 11.90 ± 0.02 μM).

This study corroborates the cytotoxic activity of the isolated compounds through the inhibition of hypoxia-inducible factor 1-alpha as well as its modulator nuclear factor kappa-light-chain-enhancer of activated B cells.

**Key words**

coumarins

sesquiterpene lactones

*Senecio graveolens*

Asteraceae

NF-κB

HIF-1α

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**Fig. S1** \textsuperscript{1}H-NMR spectrum of the *n*-heptane extract of *S. graveolens* in CDCl\textsubscript{3} 300 MHz.
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Fig. S9 $^1$H-$^{13}$C HSQC spectrum of 01SGDM in CDCl$_3$ 700 MHz.

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Fig. S11 HRMS spectrum of 01SGDM.

Fig. S12 $^1$H-NMR spectrum of 02SGDM in MeOD 700 MHz.
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Fig. S18 HRMS spectrum of 02SGDM.
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**Fig. S26** MTT cytotoxicity assays of *S. graveolens* compounds against a panel of human cancer cell lines and one noncancerous cell line after 72 h of treatment under hypoxic (1% O₂) conditions. Control = untreated cells.

**Fig. S27** LDH cytotoxicity assays of *S. graveolens* compounds against a panel of human cancer cell lines and one noncancerous cell line after 72 h of treatment under hypoxic (1% O₂) conditions. Control = untreated cells.
Table S1 Inhibitory effect of *S. graveolens* compounds on NF-κB activation in a panel of human cancer cell lines and one noncancer cell line after 72 h of treatment under hypoxic (1% O₂) conditions. Control = untreated cells.

<table>
<thead>
<tr>
<th>Samples</th>
<th>PBMCs</th>
<th>U-373 MG</th>
<th>A549</th>
<th>Hep G2</th>
<th>Caco-2</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>11.98 ± 0.01</td>
<td>12.43 ± 0.01</td>
<td>12.23 ± 0.08</td>
<td>12.45 ± 0.09</td>
<td>12.65 ± 0.08</td>
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<tr>
<td>DMSO</td>
<td>5.96 ± 0.05</td>
<td>6.35 ± 0.02</td>
<td>6.45 ± 0.03</td>
<td>6.23 ± 0.07</td>
<td>6.62 ± 0.08</td>
</tr>
<tr>
<td>JSH-23</td>
<td>7.1 ± 0.02</td>
<td>7.1 ± 0.02</td>
<td>7.1 ± 0.02</td>
<td>7.1 ± 0.02</td>
<td>7.1 ± 0.02</td>
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<tr>
<td>Compound 1</td>
<td>7.07 ± 0.01</td>
<td>7.13 ± 0.03</td>
<td>8.64 ± 0.03</td>
<td>8.87 ± 0.04</td>
<td>15.11 ± 0.01</td>
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<tr>
<td>Compound 2</td>
<td>0.41 ± 0.04</td>
<td>2.28 ± 0.01</td>
<td>3.79 ± 0.02</td>
<td>3.98 ± 0.05</td>
<td>6.41 ± 0.02</td>
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<tr>
<td>Compound 3</td>
<td>3.09 ± 0.03</td>
<td>3.62 ± 0.06</td>
<td>4.48 ± 0.03</td>
<td>5.25 ± 0.01</td>
<td>11.90 ± 0.02</td>
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Table S2 Inhibitory effect of *S. graveolens* compounds on HIF-1α in a panel of human cancer cell lines and one noncancer cell line after 72 h of treatment under hypoxic (1% O₂) conditions. Control = untreated cells.

<table>
<thead>
<tr>
<th>Samples</th>
<th>PBMCs</th>
<th>U-373 MG</th>
<th>A549</th>
<th>Hep G2</th>
<th>Caco-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>90.97 ± 0.02</td>
<td>96.71 ± 0.05</td>
<td>98.84 ± 0.05</td>
<td>97.82 ± 0.06</td>
<td>99.97 ± 0.03</td>
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<td>2-MeOE2</td>
<td>0.5 ± 0.01</td>
<td>0.5 ± 0.01</td>
<td>0.5 ± 0.01</td>
<td>0.5 ± 0.01</td>
<td>0.5 ± 0.01</td>
</tr>
<tr>
<td>Compound 1</td>
<td>11.92 ± 0.01</td>
<td>20.66 ± 0.04</td>
<td>25.80 ± 0.04</td>
<td>29.21 ± 0.03</td>
<td>38.58 ± 0.02</td>
</tr>
<tr>
<td>Compound 2</td>
<td>1.57 ± 0.04</td>
<td>2.29 ± 0.07</td>
<td>4.13 ± 0.04</td>
<td>6.40 ± 0.03</td>
<td>9.80 ± 0.04</td>
</tr>
<tr>
<td>Compound 3</td>
<td>10.22 ± 0.03</td>
<td>15.22 ± 0.01</td>
<td>17.47 ± 0.02</td>
<td>18.26 ± 0.06</td>
<td>19.75 ± 0.04</td>
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