

Supplementary Information

Molecular genetic analysis of the cichorine gene cluster in *A. nidulans*

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Supplementary Table 1. Primers Used in This Study.

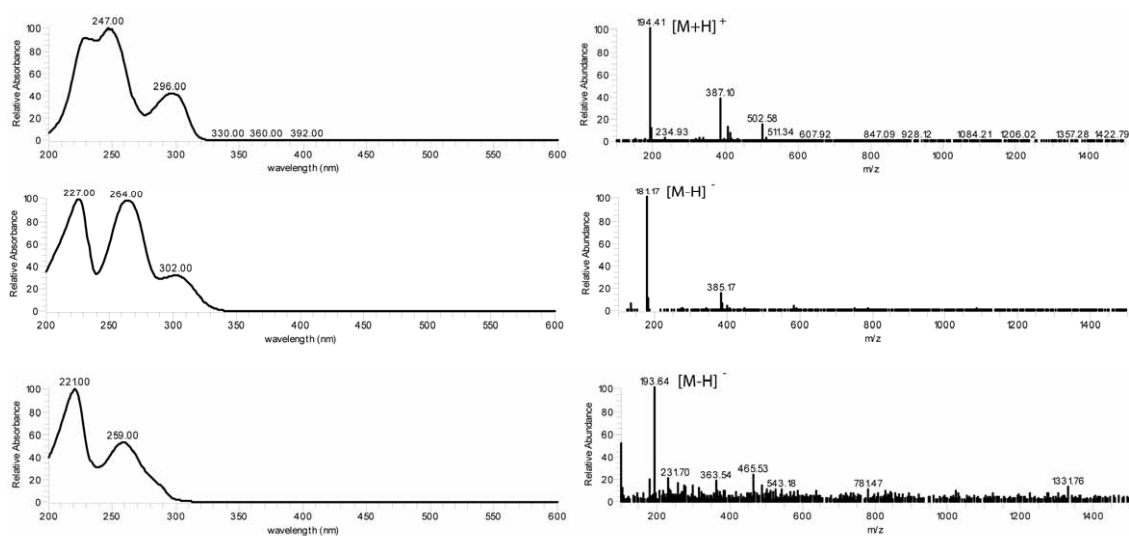
AN11921	<i>cicA</i>
AN11921P1: GCT ACA ACC AGT ATC CGC AC	<i>cicAP1</i> : TCA ACG AGC ATA TTC CAG CC
AN11921P2: CTG TTC TAC CTC CAC CAT GC	<i>cicAP2</i> : ATC ACT GCG GAT CGG AAG AC
AN11921P3: *CTG GGG TTG TTG AAA TCG AGT	<i>cicAP3</i> : *AGT CTA GCC GAT GCT TTT GC
AN11921P4: **GAC GAC GTT TCT CGT TCT TTG	<i>cicAP4</i> : **ATT GGA TGG AGT GAG GTT GG
AN11921P5: CAT CGG AAT CGT CGG ACG AAC	<i>cicAP5</i> : TGG TGT TGA GAG CAC CAA TG
AN11921P6: CAT TTG ACA ACG TCG CCA TGC	<i>cicAP6</i> : CGT CTA TCA TGC TTG CCA TTG
<i>cicB</i>	<i>cicC</i>
<i>cicBP1</i> : GAT GTG ACA AAG TTC CAA TCG	<i>cicCP1</i> : CAA TGA TGG CTG CTG CAG AG
<i>cicBP2</i> : GTG ACA AAG TTC CAA TCG ACA AG	<i>cicCP2</i> : AGG AGA CTG TTG ATA GCA GG
<i>cicBP3</i> : *GTT TCG AGC CAT GGC TGA G	<i>cicCP3</i> : *CGC GTT CTT TTG TTG CGA TG
<i>cicBP4</i> : **TGC GGT TGC TAG ACG TTA TC	<i>cicCP4</i> : **ATC TTT CAC ATT TGT GGG CTC
<i>cicBP5</i> : CAG ATA TCC TGC AAT TCC AGC	<i>cicCP5</i> : TAC CAG CCG TAA TCA TGA GG
<i>cicBP6</i> : CTT CGT CAA AAC GTC GAC C	<i>cicCP6</i> : GCA TGG GAG ATA CCA GAC TG
<i>cicD</i>	<i>cicE</i>
<i>cicDP1</i> : CAT GAT GGT CTC ACG TGT GC	<i>cicEP1</i> : CAA CCG ACA CTG CTG TGA G
<i>cicDP2</i> : TGA CGA ACT GCA GGT AAG AAG	<i>cicEP2</i> : CGA ACG GCT CTG AGA ACA TC
<i>cicDP3</i> : *GAG GTA TTC TGG AAG G	<i>cicEP3</i> : *GAA GCG TTC CAT GAT GGT CT
<i>cicDP4</i> : **GAG GAA TCG GCA AGT TCT TG	<i>cicEP4</i> : **GGT GTC TGT CCA CTT GTT G
<i>cicDP5</i> : GAT GAG ATC GTC GAG TGG CT	<i>cicEP5</i> : GAA TTT AGC CAC GGC TTT GC
<i>cicDP6</i> : CGA TGG CTG GCA TTA CGC TT	<i>cicEP6</i> : GAG AAG TTC GTA CTG AAA GCC
<i>pkbA</i>	<i>cicG</i>
<i>pkbAP1</i> : GCT CAC AGT ATG ATT TGG ATC	<i>cicGP1</i> : ATC AGA AGA CGC AGC CTC G
<i>pkbAP2</i> : GGA AAG CAC TTG GAA GCA TAC	<i>cicGP2</i> : GAC TAG GGT CGT CCA TTT GC
<i>pkbAP3</i> : *CGG AGC CAT GAT GAA AGA AC	<i>cicGP3</i> : *GTT AAT CGA TGA AAT TGG CAC C
<i>pkbAP4</i> : **ACC TAG TCA TCT CGC TTA GAG	<i>cicGP4</i> : **AGA GAT CAA GAC ATG CGT GC
<i>pkbAP5</i> : GAC GAC GTG AAC AGC CTA GT	<i>cicGP5</i> : GTG CAG ACG CAG ATA CCT G
<i>pkbAP6</i> : CCT CTC CAT GGT AAA TGC TG	<i>cicGP6</i> : CTG TGC ATC GTG ACC GTG A
<i>cicH</i>	AN6450
<i>cicHP1</i> : CAG ACA TTG ATC GCC TGG TG	AN6450P1: CTA TCC ATC AAG TCG AGT CAG
<i>cicHP2</i> : TCG GCA AGA TCG ACA TCC TC	AN6450P2: TTC TGC ATA TCA GCG CTT CC
<i>cicHP3</i> : *CTC GGT ACC AAT GCA ACA CA	AN6450P3: *ACT AGC TGT AGA GGG AGA TTA G
<i>cicHP4</i> : **AAA GGG AGG GCT GAT CTG TAG	AN6450P4: **AGA GGA CGC TGG TCT TTT GG
<i>cicHP5</i> : CCC CTC TTA ATT GGA CAT GTC	AN6450P5: CGA AAT TAC AGA CCT TGC CAG
<i>cicHP6</i> : GGT TTC TTC TCA TGG ATT CCT	AN6450P6: AGT CGT CTG GAG AAT GTG ATC

*All P3 sequences begin with CGA AGA GGG TGA AGA GCA TTG.
**All P4 sequences begin with GCA TCA GTG CCT CCT CTC AGA CAG.
These P3/P4 beginning sequences are tails that anneal to the *A. fumigatus* pyrG fragment (AfpyrG) during fusion PCR.

Supplementary Methods

NMR spectra were run on a Varian Mercury Plus 400 spectrometer. IR spectra were obtained on a Bruker Vertex 80 FTIR spectrometer. For all structural determinations twenty-five 150x15 mm petri dishes containing solid YES media were inoculated with an *A. nidulans* strain. All compounds, from crude extract, were initially applied to a SiO₂ column (Merck 230-400 mesh, ASTM) and eluted with 1 L fractions. All collected fractions were further purified by reverse phase HPLC [Phenomenex Luna 5 μ m C18 (2), 250 x 10 mm] with a flow rate of 10.0 mL/min and measured by a UV detector at 250 nm. Solvent A was 5% acetonitrile in water with 0.05% trifluoroacetic acid. Solvent B was 100% acetonitrile with 0.05% trifluoroacetic acid.

For the isolation of compounds **1**, **5**, and **6** LO2026, *cicBA* Δ , and *cicCA* Δ , respectively were cultivated. For **1** and **5** 2:98 methanol:dichloromethane was the eluent for silica gel chromatography, and for **6** 100% dichloromethane was the eluent. The HPLC gradient system was 10 to 33% B from 0 to 23 min, 33 to 100% B from 23 to 25 min, 100% B from 25 to 28 min, 100% B to 10% B from 28 to 30 min, and re-equilibration to 10% B from 30 to 33 min.



Supplementary Figure 1. UV and ESIMS profiles of **1** ($m/z = 193$), **5** ($m/z = 182$), and **6** ($m/z = 194$).