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Synthesis and evaluation of novel ellipticines and derivatives as fungicides and inhibitors of *Phytophthora infestans*

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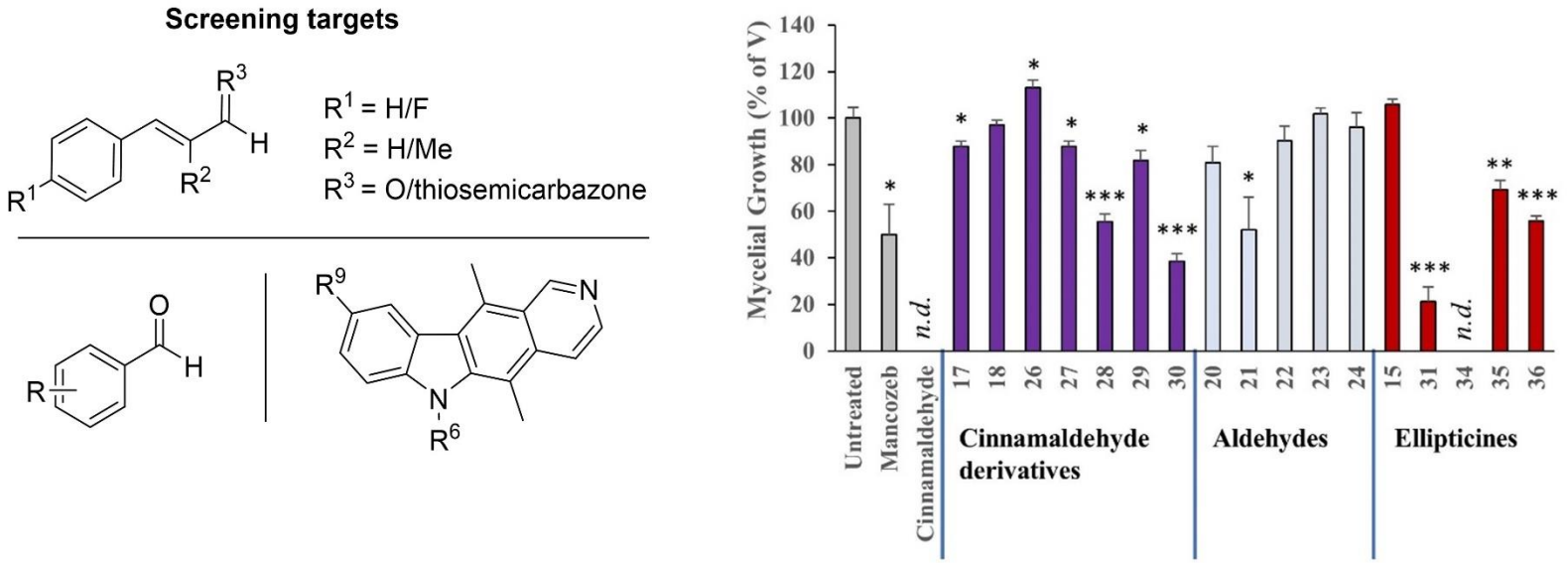
Synthesis and evaluation of novel ellipticines and derivatives as fungicides and inhibitors of *Phytophthora infestans*

Graphical Abstract



Structure of screening targets and the effects of compounds on *P.infestans* mycelial growth after 5 days

Abstract: The pathogen *Phytophthora infestans* is responsible for worldwide catastrophic crop damage and discovery of new inhibitors of this organism is of paramount agricultural and industrial importance. Current strategies for crop treatment are inadequate with limitations of efficacy and market alternatives. Ellipticines have recently been discovered to have fungicidal properties and have been assessed against *P. infestans* growth with promising results. We hereby report a probe of the ellipticine framework to investigate the alkyl subunit and screen a set ellipticines and derivatives to identify new lead compounds to act against *P. infestans*. A series of ellipticinium salt derivatives have been identified with exceptional growth inhibitory activity and apparent lack of toxicity towards a human cell-line, surpassing the effect of known and marketed fungicides. This report identifies the potential of this natural product derivative as a novel fungicide.

Keywords: Ellipticine; fungicide; phytophthora infestans

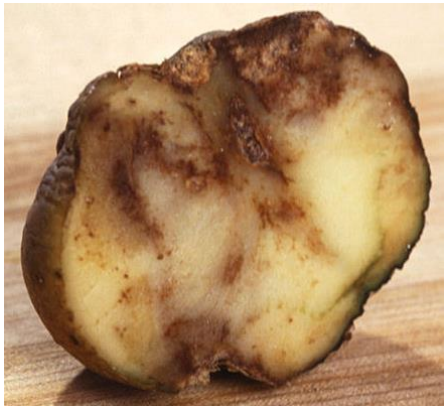
Mackrill, J.J.; Kehoe, R.A.; Zheng, L.; McKee, M.L.; O'Sullivan, E.C.; Prestwich, B.M.D.; McCarthy, F.O. Inhibitory Properties of Aldehydes and Related Compounds against Phytophthora infestans—Identification of a New Lead. Pathogens 2020, 9, 542. https://doi.org/10.3390/pathogens9070542

McKee, M.L.; Zheng, L.; O'Sullivan, E.C.; Kehoe, R.A.; Doyle Prestwich, B.M.; Mackrill, J.J.; McCarthy, F.O. Synthesis and Evaluation of Novel Ellipticines and Derivatives as Inhibitors of Phytophthora infestans. Pathogens 2020, 9, 558. https://doi.org/10.3390/pathogens9070558

Introduction

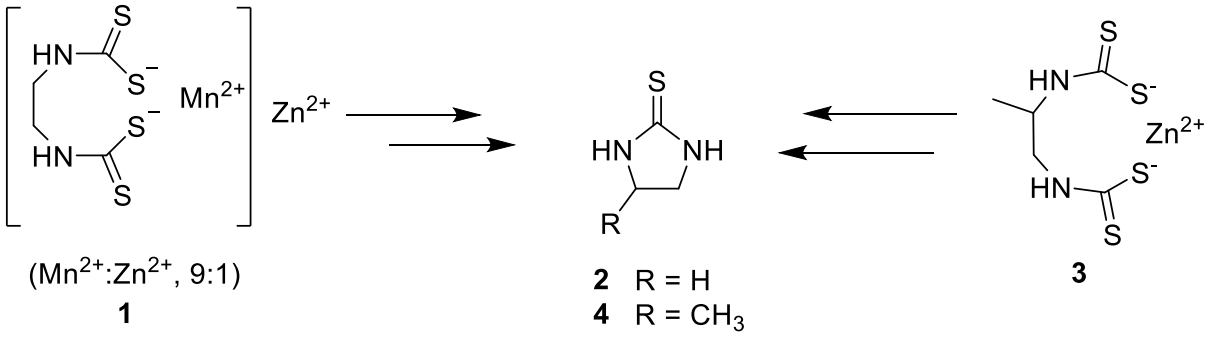
- *Phytophthora infestans* is responsible for worldwide catastrophic crop damage
- First described by Berkeley in 1846 (aptly translates as “plant destroyer”)
- Irish potato famine in 1845–1849, resulting in the deaths of one million people and a further one million emigrating

- Global impact is in excess of $6.2 billion per annum due to crop loss/fungicides use
- Resistant strains of *P. infestans* continually developing
- Designing novel fungicides to target this pathogen is of high importance for food sustainability and agriculture



Fungicides

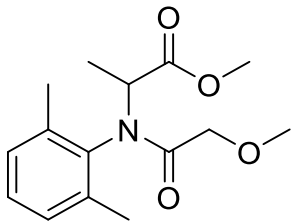
- Dithiocarbamate class of fungicides dates from the 1960s
  - Mancozeb **1** and Propineb **3** are two of the most widely used
  - Both organosulfur fungicides found to break down into toxic metabolites **2**, **4**
  - Propineb **3** has been banned by the European Commission and Mancozeb **1** is now under scrutiny for its negative effects



- Resistance is a problem
- Significant and immediate need for effective replacements

Fungicides

- Phenylamide fungicides developed in the 1970s: Metalaxyl **5** one of the most popular
- By 1980, resistant isolates of *P. infestans* were discovered worldwide



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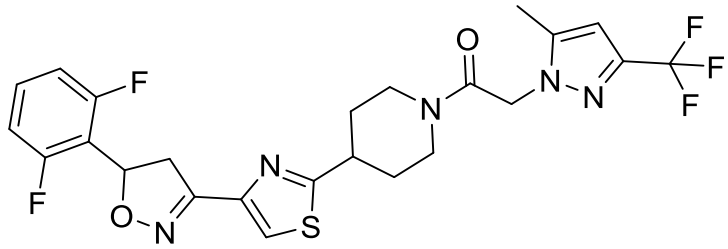

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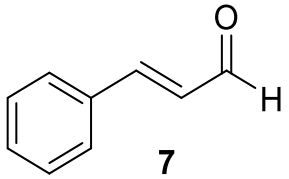
- Developed in 2016 and approved by the EU in 2017, oxathiapiprolin **6** has been used in more recent years as a targeted antioomycete fungicide

- Already been found to be ineffective against emerging resistant strains of *Phytophthora capsici*

Alternative Strategies

- Natural products as fungicides
- Cinnamaldehyde **7** has evident fungicidal activity



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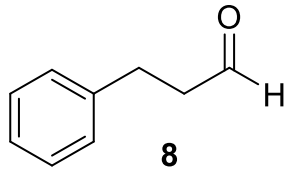

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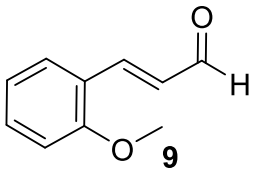
- Cinnamaldehyde analogues subjected to *P. infestans* testing have retained their aldehyde functionality and are substituted on the aromatic ring (**9**-**13**)



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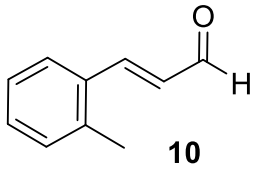

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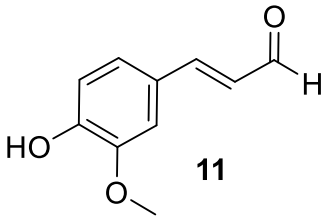

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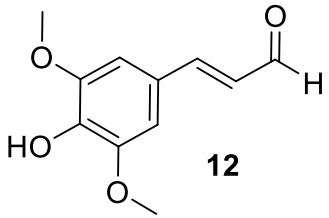

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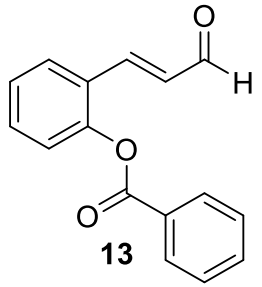

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- To test the theory that the α, β-unsaturated bond in cinnamaldehyde is important, Hu *et al.* monitored Ca^{2+} levels using cinnamaldehyde and a derivative without the α, β-unsaturated bond, hydrocinnamaldehyde **8**.

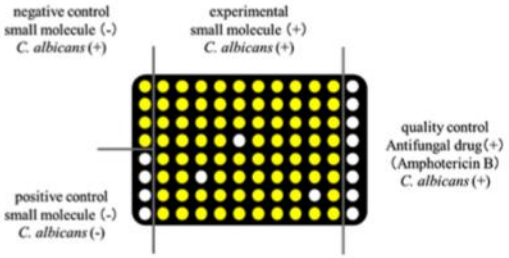
- Treatment of *P. capsici* with hydrocinnamaldehyde **8** demonstrated no inhibitory effects of zoospore growth indicating that the Michael addition mechanism was essential

PLoS ONE 2013, 8, e76264–e76264

Related Fungicides

- A recent study by Watamoto found a diverse set of compounds to demonstrate inhibitory effects on fungal strains, amongst which some of the structural features of our targets align
- Hit compound comparison on Candida biofilm formation

- Michael acceptors: Bay 11-7082 possesses an α, β-unsaturated bond similar to cinnamaldehyde and may act through a similar mechanism.
- Ellipticine has known antimicrobial and especially anticancer properties
- Quaternary salts and long chain aliphatic amines also seen
- Could these be related or combined?



hit compound	percent inhibition
Bay 11-7085	100
Chelerythrine chloride	82.4
Dequalinium chloride hydrate	71.5
Ellipticine	100
Indatraline hydrochloride	85.2
Palmitoyl-DL-carnitine chloride	76.0
Bay 11-7082	100
Sanguinarine chloride hydrate	100
CV-3988	93.3

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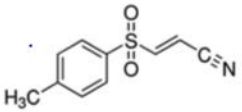
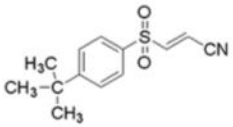
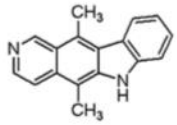


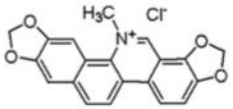
Bay 11-7082 (MW: 207.3)
An inhibitor of IκB-α phosphorylation by cytokines



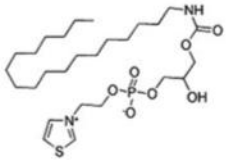
Bay 11-7085 (MW: 249.3)
Repress ICAM-1, VCAM-1, E-selectin, IL-6 and IL-8 activated by NF-κB



Ellipticine (MW: 246.3)
Ellipticine is an antitumor alkaloid isolated from *Ochrosia* spp. It inhibits cytochrome P450 (CYP1A1) and DNA topoisomerase II activities



Sanguinarine chloride hydrate (MW: 367.8)
Natural product that has antibacterial, antiinflammatory and antioxidant effect. Anti-proliferation and apoptosis promoting effect against some cancer cells.

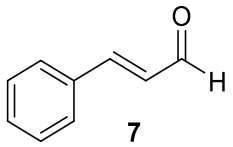


CV-3988 (MW: 592.8)
Competitive PAF receptor antagonist. Inhibits PAF-induced human platelet aggregation (3-30 μM) and bronchoconstriction in the guinea pig.

Hirofumi Y., Frontiers of Micro., 2015, 6, 1453

Our initial probe into effect on *P. infestans* mycelial growth

- Initial test on mycelial growth for **cinnamaldehyde**, **hydrocinnamaldehyde** and **mancozeb** to validate assay
- Expected effects seen for Cinnamaldehyde at high doses
- Hydrocinnamaldehyde also shows inhibition albeit at higher concentration and contrasts with earlier work in the field
- Mancozeb **1** is significantly more potent
- Signals there is room for improvement and structural exploration



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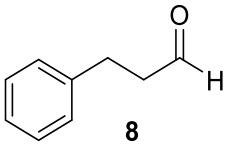

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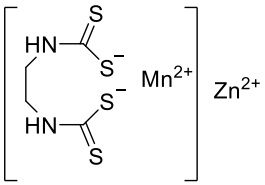
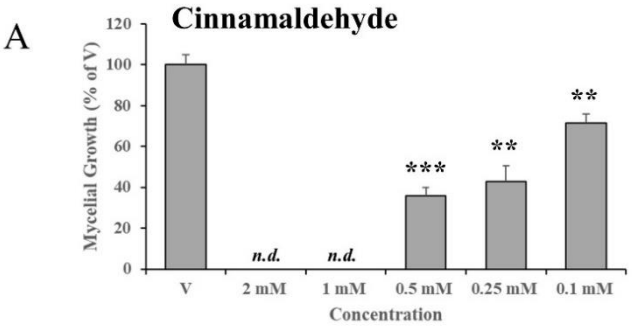
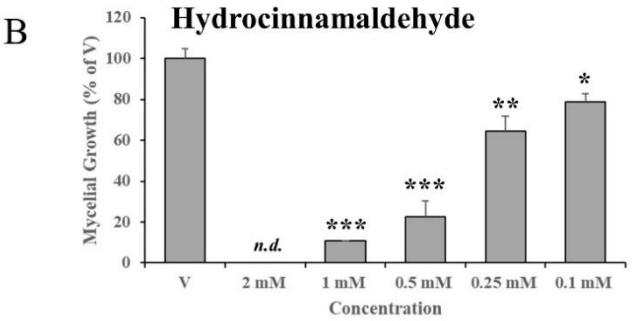
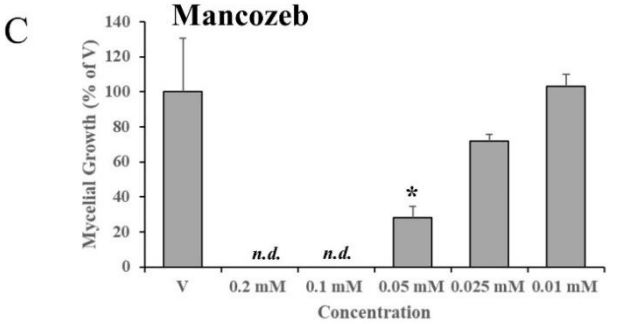


(Mn^{2+}:Zn^{2+}, 9:1)

1



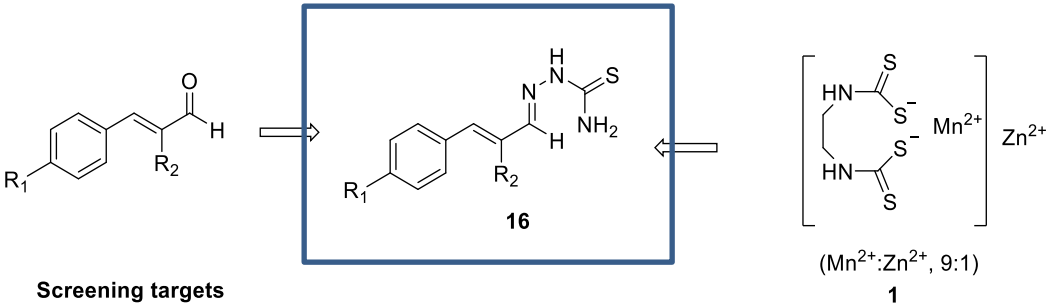




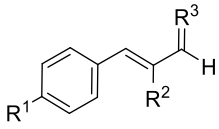
Pathogens 2020, 9, 542. https://doi.org/10.3390/pathogens9070542

Defining a screening set for evaluation

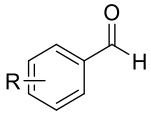
- Consider the possibility of fragment combination
- Screening targets from cinnamaldehydes, aldehydes thioureas and ellipticines
- Cinnamaldehyde set to test steric and electronic influence
- Modification of cinnamaldehyde to screen thiourea and conjugation
- Aldehyde screen to test new potential leads
- Ellipticine and ellipticine aldehydes

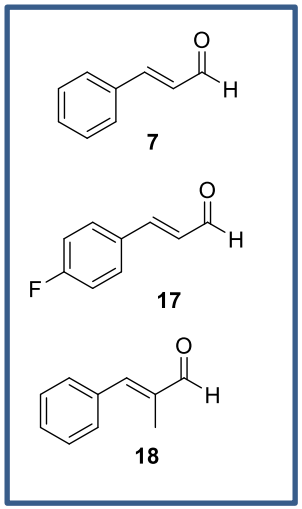


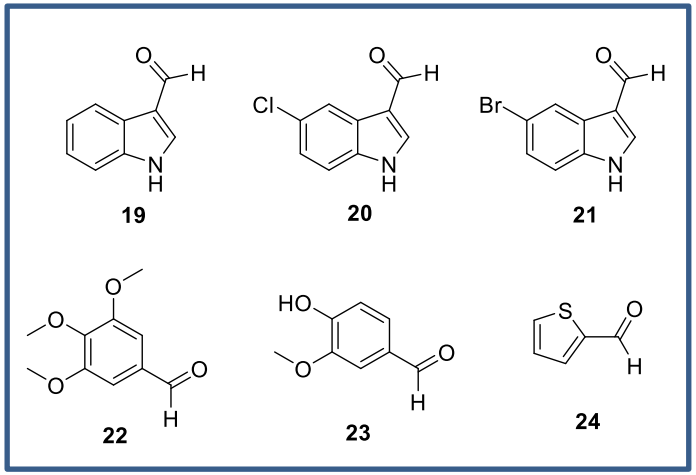
Screening targets



R^1 = H/F
R^2 = H/Me
R^3 = O/thiosemicarbazone

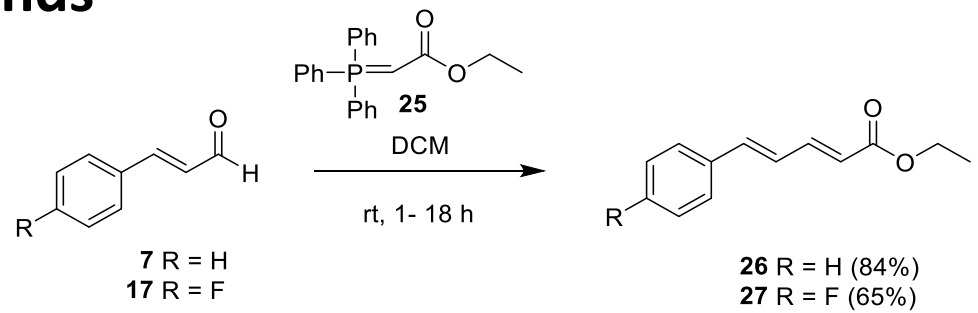






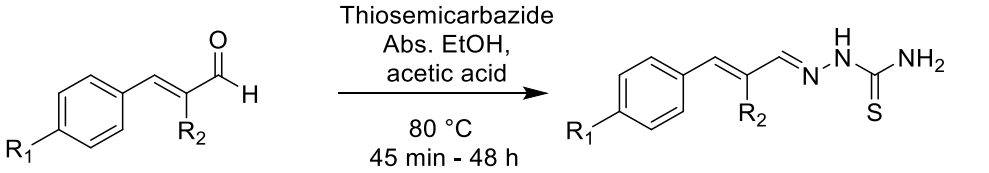
Synthesis of new probe compounds

- Screening targets from cinnamaldehydes, aldehydes thioureas and ellipticines
- Cinnamaldehyde set to test steric and electronic influence
- Modification of cinnamaldehyde to screen thiourea and conjugation
- Ellipticine and ellipticine aldehydes



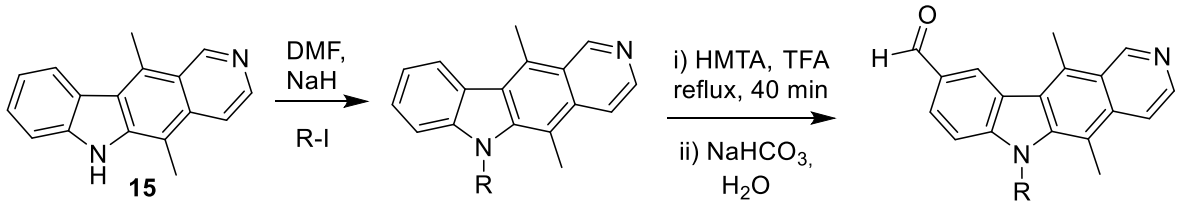
7 R = H
17 R = F

26 R = H (84%)
27 R = F (65%)



7 R_1 = H, R_2 = H
17 R_1 = F, R_2 = H
18 R_1 = H, R_2 = Me

28 R_1 = H, R_2 = H (34%)
29 R_1 = F, R_2 = H (60%)
30 R_1 = H, R_2 = Me (51%)



31 R= CH_3 (92%)
32 R= CH_2CH_3 (75%)
33 R = *i*Pr (36%)

34 R= CH_3 (81%)
35 R= CH_2CH_3 (90%)
36 R = *i*Pr (94%)

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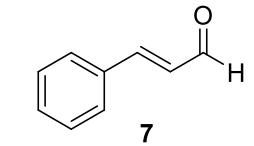
Screen of the effects of compounds on *P. infestans* mycelial growth after 5/9/13 days at 25µM



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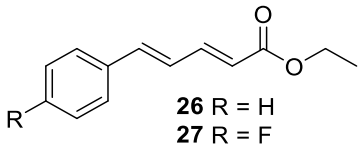
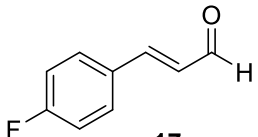


26 R = H
27 R = F



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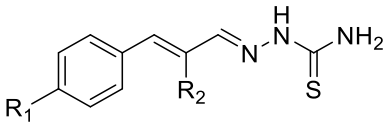
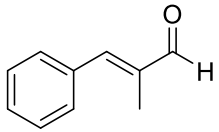


28 R_1 = H, R_2 = H
29 R_1 = F, R_2 = H
30 R_1 = H, R_2 = Me



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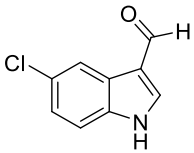

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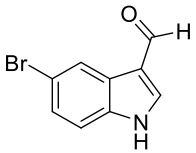

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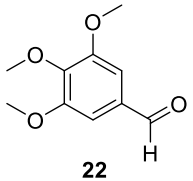

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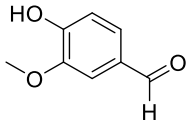

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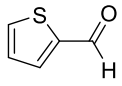

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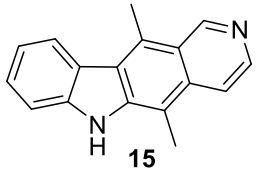

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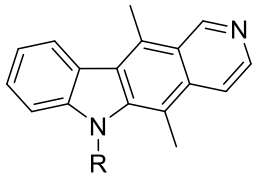
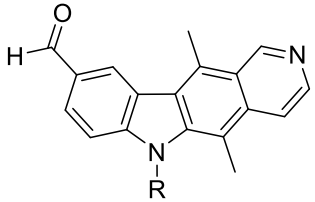
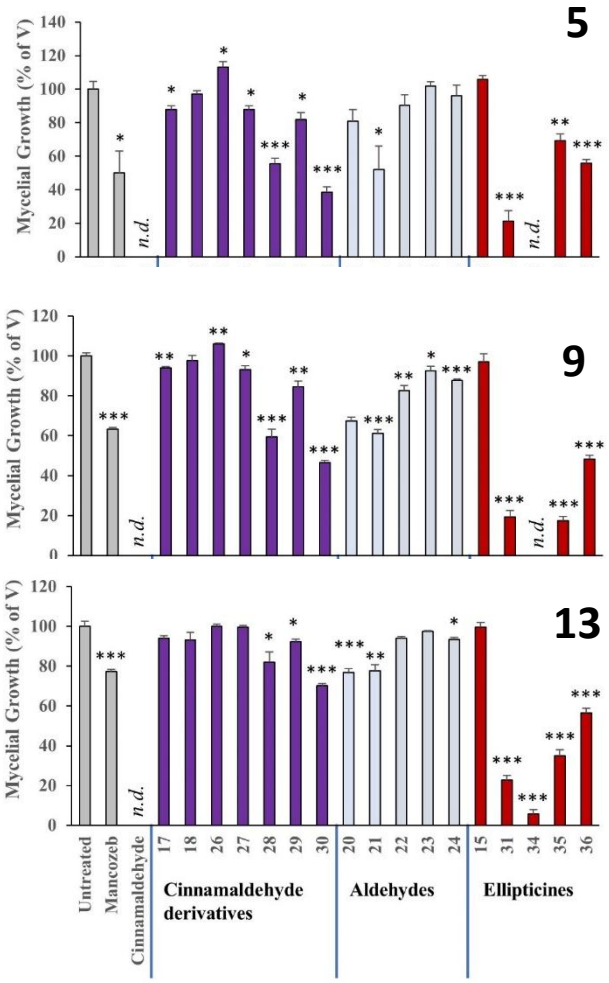


31 R= CH_3



34 R= CH_3
35 R= CH_2CH_3
36 R = *i*Pr



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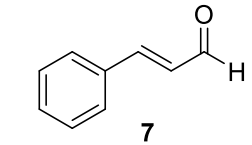
Effect of test compounds on *P. infestans* zoosporogenesis/ sporangiogenesis at 25µM



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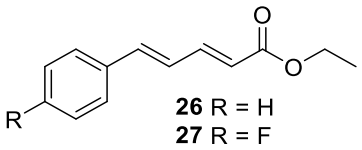
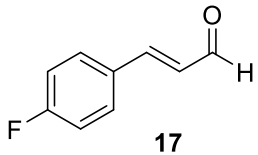


26 R = H
27 R = F



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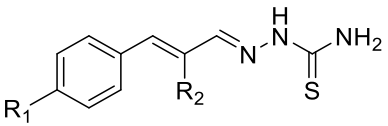
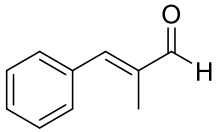


28 R_1 = H, R_2 = H
29 R_1 = F, R_2 = H
30 R_1 = H, R_2 = Me



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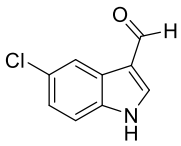

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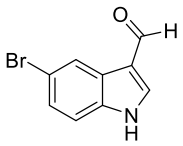

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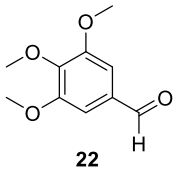

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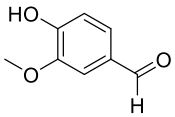

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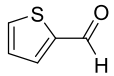

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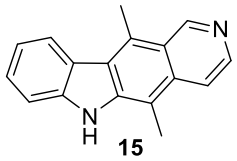

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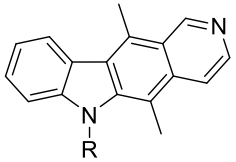
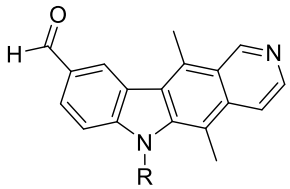
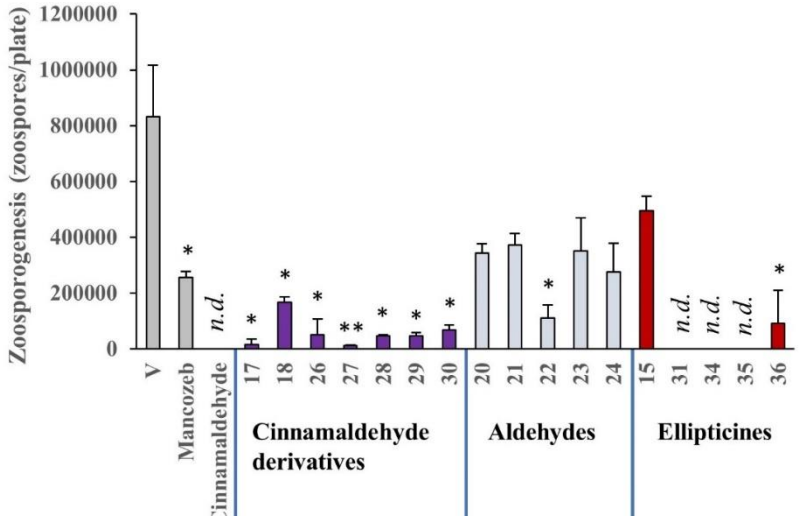
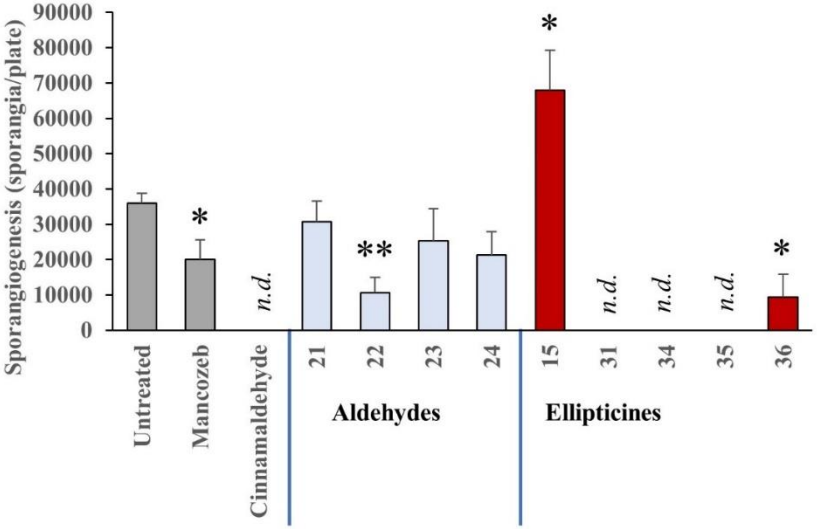


31 R= CH_3



34 R= CH_3
35 R= CH_2CH_3
36 R = *i*Pr





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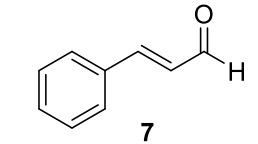
Effect of test compounds on reduction of XTT by a human cell line, HEK-293T at 25µM



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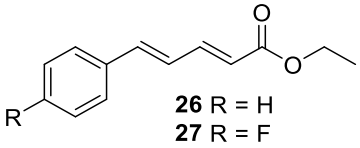
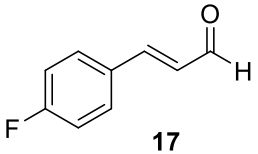


26 R = H
27 R = F



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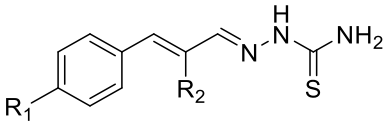
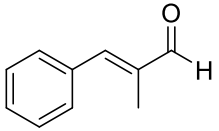


28 R_1 = H, R_2 = H
29 R_1 = F, R_2 = H
30 R_1 = H, R_2 = Me



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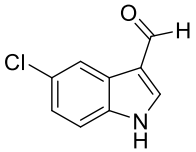

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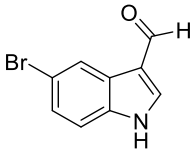

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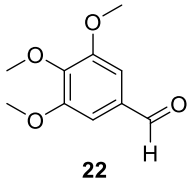

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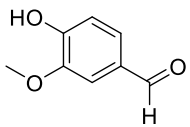

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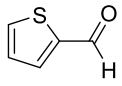

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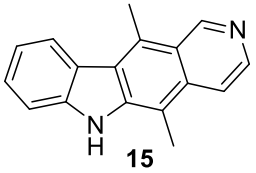

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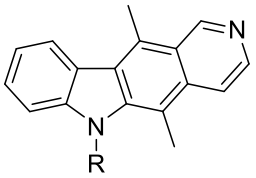
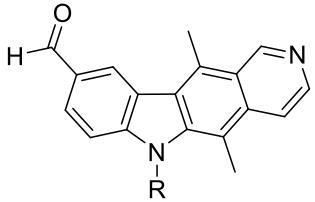
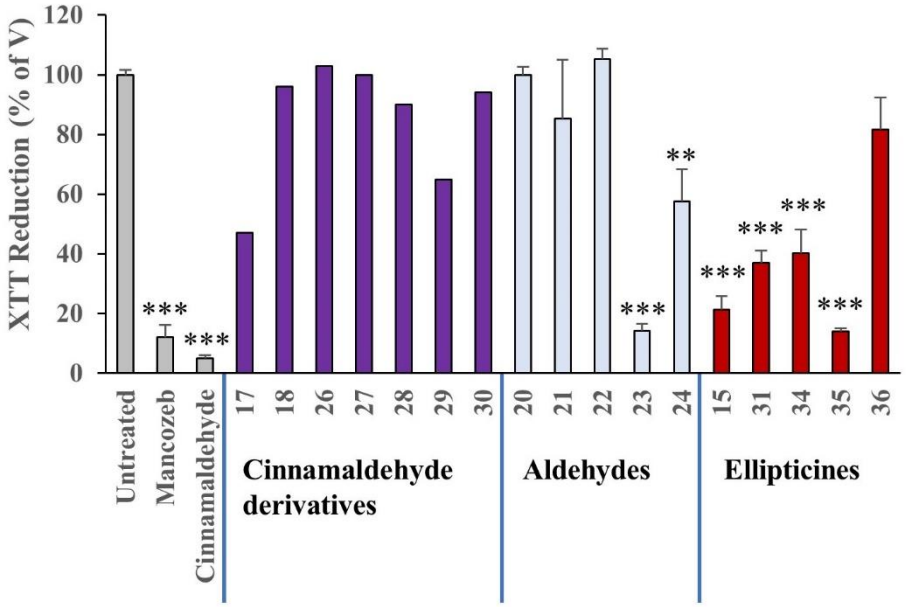


31 R= CH_3



34 R= CH_3
35 R= CH_2CH_3
36 R = *i*Pr



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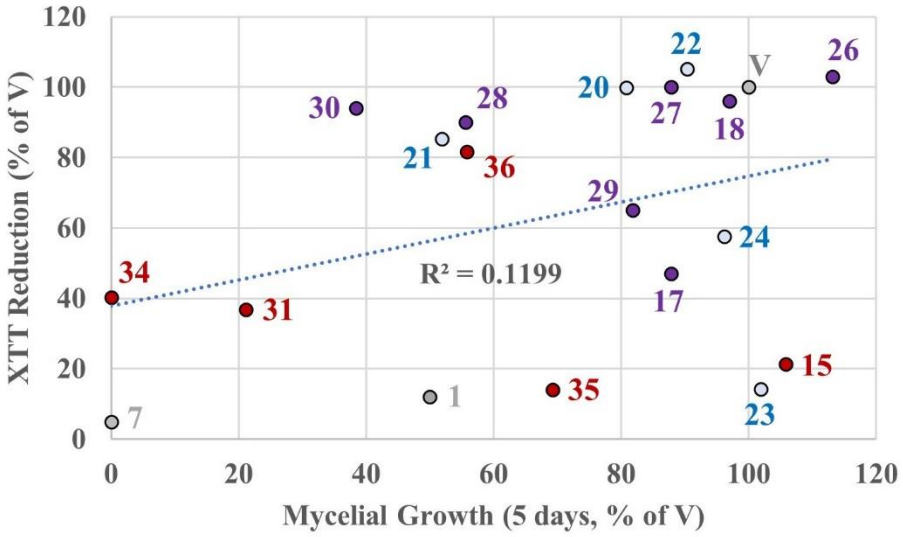
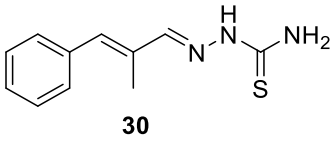
Mycelial growth Vs toxicity





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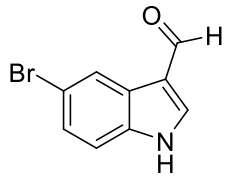

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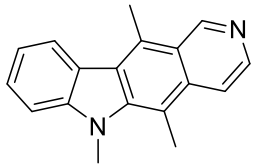

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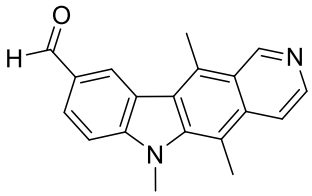

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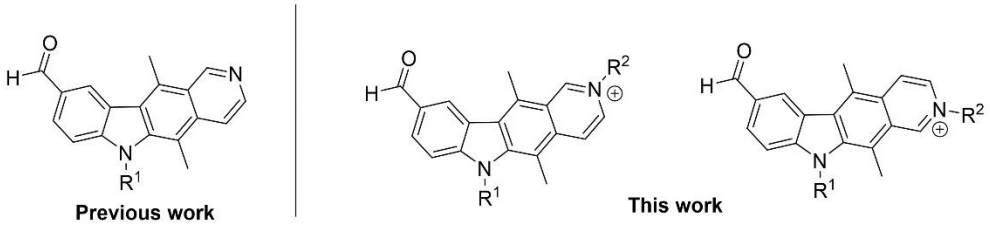
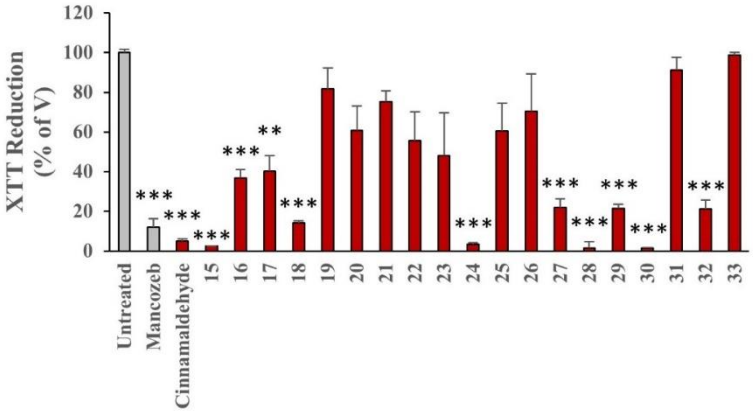
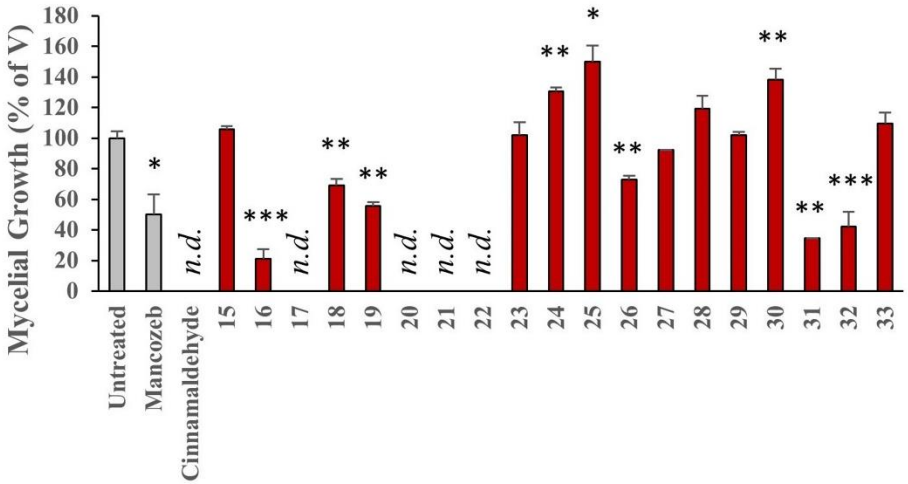
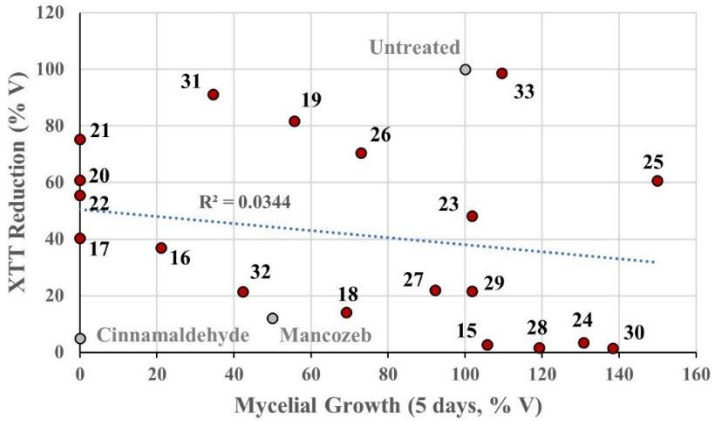
Looking futher into ellipticines….









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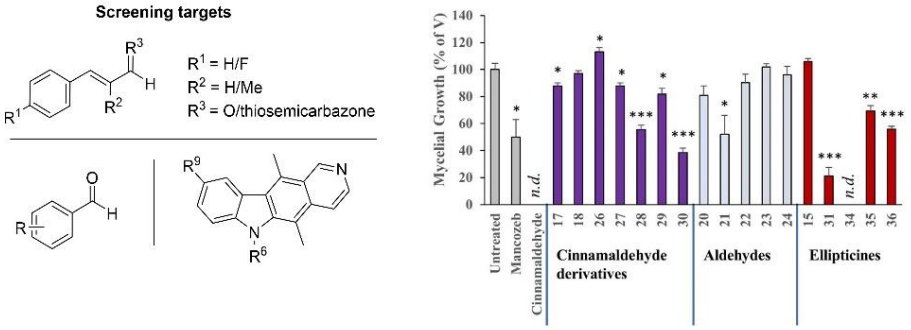
Conclusions

- Screened a library of cinnamaldehydes, aromatic aldehydes and ellipticines for inhibition of *P. Infestans* growth over a period of 2 weeks
- Ellipticine derivatives which incorporate an aldehyde have been identified with exceptional growth inhibitory activity and apparent lack of toxicity towards a human cell-line surpassing the effect of known and marketed fungicides.



Structure of screening targets and the effects of compounds on *P.infestans* mycelial growth after 5 days

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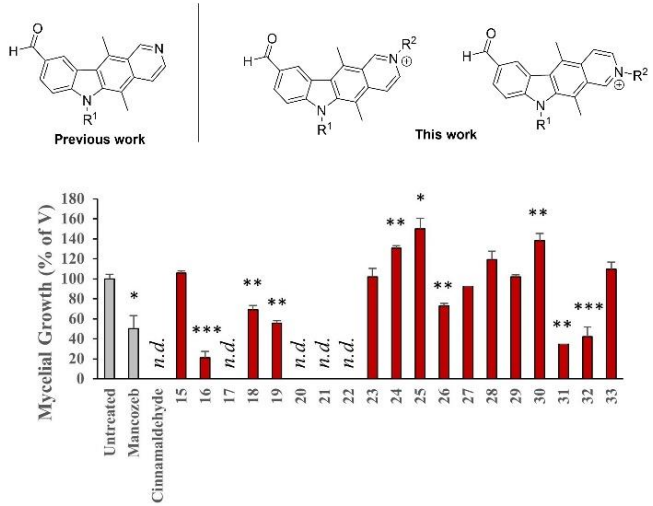

Pathogens 2020, 9, 558. https://doi.org/10.3390/pathogens9070558

- This report identifies the potential of this natural product derivative as a novel fungicide

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- Dr John Mackrill and Dr Limian Zheng
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  - Irish Research Council
- National Cancer Institute