HHS Public Access

Author manuscript

Cell Rep Phys Sci. Author manuscript; available in PMC 2021 October 01.

Published in final edited form as:

Cell Rep Phys Sci. 2021 September 22; 2(9): . doi:10.1016/j.xcrp.2021.100548.

Photochemically derived 1-aminonorbornanes provide structurally unique succinate dehydrogenase inhibitors with *in vitro* and *in planta* activity

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SUMMARY

Agrochemical fungicidal leads have been prepared from photochemically derived 1-aminonorbornane building blocks. The unique 1-aminonorbornane core is generated via direct excitation of a Schiff base precursor, leveraging the *N*-centered radical character of the excited state species to facilitate a series of radical reactions that construct the norbornane core. This process requires no exogenous reagents, only solvent and photons; thus, it represents an exceptionally simple and efficient means of generating the key building blocks. These (hetero) arene-fused 1-aminonorbornanes are unprecedented in both the agrochemical and pharmaceutical discovery literature; therefore, photochemical advances have provided the unique opportunity to explore the functional utility of novel chemical space. Toward this end, the 1-aminonorbornanes were used to generate next-generation succinate dehydrogenase inhibitors. *In vitro* fungicidal activity is demonstrated against three fungal plant pathogens affecting field crops, specifically: *Fusarium graminearum*, *Sclerotinia sclerotiorum*, and *Macrophomina phaseolina*. The *in vitro* performance against *F. graminearum* was shown to translate into a greenhouse setting. The discovery of *in planta* fungicidal activity illustrates the interdisciplinary value available via photochemical innovation.

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AUTHOR CONTRIBUTIONS

D.S., J.L.C., and R.C.M. performed the synthetic experiments; M.B., V.O., and H.S. performed the *in vitro* experiments; D.S. and M.B. performed the *in planta* experiments; D.S., M.B., M.I.C., and C.R.J.S. designed the experiments; D.S., M.B., V.O., H.S., J.L.C., M.I.C., and C.R.J.S. wrote the manuscript.

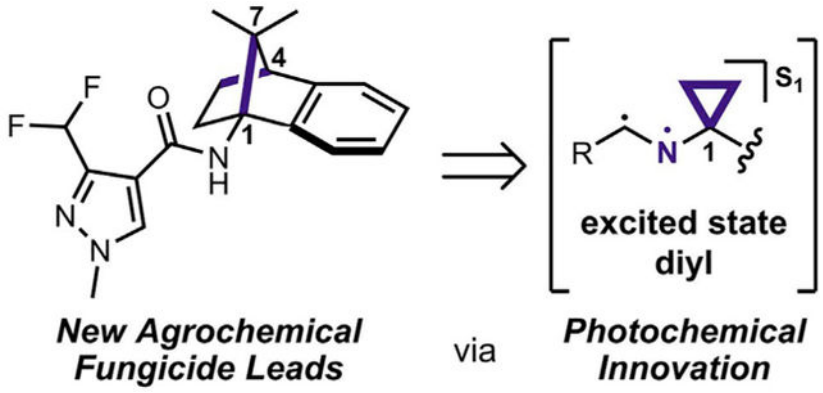
SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrp.2021.100548.

DECLARATION OF INTERESTS

Efforts related to this work are the subject of ongoing commercialization efforts, as supported by the University of Michigan Office of Technology Transfer. D.S. and C.R.J.S. are authors of provisional patent filings associated with this research.

Graphical Abstract



Advances in synthetic photochemistry can directly affect the world of agrochemical development. Staveness et al. report using photochemically derived 1-aminonorbornanes to produce and evaluate a series of fungicidal candidates *in vitro* and in the greenhouse and that offer fungicidal activity on par with commercial agents.

INTRODUCTION

Advances in synthetic photochemistry have disruptive potential to influence modern industrial chemistry.[1,2] Excited state species offer reactivity modes that are fundamentally unique, in regard to both the reactive intermediates involved and the structural motifs that can be generated.[3,4] In many cases, photochemical methodology offers the most efficient, if not the only, reliable means of preparing a given scaffold. Strained, saturated carbocycles and heterocycles are arguably the most prominent demonstration of this concept, evidenced by classic examples such as the synthesis of oxetanes via the Paterno-Buchi reaction or cyclobutanes via the De Mayo [2+2] photocycloaddition. This attribute of photochemistry is optimally aligned with the rising prominence of sp^3-centric drug design[5] and the pressing need for increased diversification of synthetically accessible chemical space.[6,7] This alignment explicitly communicates the opportunity to affect the industrial sector via a concerted focus on photochemical reaction science.

Importantly, while most synthetic labs frame the applications of their work around pharmaceutical development, the opportunity to influence agrochemical development is equally advantageous. Agrochemistry faces many of the hallmark challenges of medicinal chemistry (e.g., target specificity, biodistribution, genetic variation in the target population),[8] and the ability to explore new chemical space necessarily improves the likelihood of designing, identifying, and optimizing for new functions in either setting. One could argue that delivering efficient synthetic access to novel molecular frameworks is even more imperative for the agrochemical sector, given the exceedingly stringent cost-of-production restrictions (generally ≤$200/kg).

In regard to fungicidal development, the most pressing concern for the field is the rising resistance pressures.[9] Fungal pathogens are developing resistance at alarming rates, and

there is a clear need for next-generation fungicides with differentiated activity, as current commercial agents become less effective in the face of increasingly prominent resistant strains. Achieving highly effective control of fungal pathogens is of paramount importance to ensure prolonged food security.[10] With the global population projected to reach 9 billion by 2050, food production must rise 60%–100% in that time frame.[11] Fungal pathogens are already estimated to cause a 30% loss across the production chain (e.g., harvest, storage, distribution),[12] which does not account for downstream effects of mycotoxin contamination in livestock, aquaculture, and dairy industries. The mid-1990s outbreak of *Fusarium graminearum* (wheat head blight) in North Dakota illustrates the economic impact that can result from uncontrolled fungal pathogens, eliciting estimated losses of $2.5 billion in the grain sector and $7.7 billion across the state economy after accounting for secondary market effects.[13] Unsurprisingly, *F. graminearum* was recognized as one of the "top 10 most dangerous fungal pathogens" in modern agriculture.[14,15] Furthermore, climate change is anticipated to exacerbate fungal diseases as well as change the geographic distribution of disease occurrence.[10] While small-molecule fungicides clearly are not the only disease management options moving forward (genetically engineered crops, increased breeding efforts, biofungicides, improved crop rotation and cultural practices), they are assuredly a necessary component of the multifaceted solution that will be needed to achieve sustainable food security in this era of rapidly emerging challenges.

As mentioned above, new photochemical methodology is well suited to rapidly translate from academic lab benches to leading edge discovery and development efforts. Of the many potential interdisciplinary applications for this new reaction science, fungicidal agents (and other agrochemicals) should be a key focus; accessing new chemical space and generating novel fungicidal leads is an exceptionally facile means of influencing industrial chemistry and eliciting real-world impact. The effort reported herein is our preliminary progress toward this goal, detailing the synthesis and evaluation (both *in vitro* and *in planta*) of 1-aminonorbornane-based succinate dehydrogenase inhibitors (SDHIs) as novel fungicidal leads for the control of *Ascomycete* plant pathogens.

RESULTS AND DISCUSSION

Preparation of SDHI candidates

The design and preparation of the fungicidal candidate library detailed herein was founded on an enabling photochemical methodology recently developed in the Stephenson lab.[16] The unique (hetero)aryl-fused 1-aminonorbornane (1-aminoNB) scaffold was accessed through a masked *N*-centered radical strategy, in which direct irradiation of cyclopropylimine starting materials engaged in formal intramolecular [3+2] cycloadditions to forge the norbornane core (see Figure 1). More specifically, *N*-cyclopropyl 4-nitrobenzimines with an appropriately tethered styrene-like motif were irradiated with 390 nm light to facilitate an $n \rightarrow \pi^*$ transition. From the $S_1(n,\pi^*)$ state, the *N*-centered radical character drives the homolytic fragmentation of the cyclopropane to initiate the requisite *6-exo-trig*, *5-exo-trig* radical cyclizations, returning the radical character to the nitrogen and thus facilitating the termination of both radical intermediates through simple reformation of the imine motif. The operational simplicity of this photochemical method provides rapid entry to the protected 1-

aminoNB scaffolds, and simple solvolysis of the Schiff base was shown to readily generate the free amine for *N*-derivatization.

These 1-aminoNB building blocks were directed toward the SDHI class of fungicides owing to the close analogy to design features within this class. Initially brought to market in 2003 with the introduction of boscalid, SDHIs are characterized by a carboxamide pharmacophore appended to a hydrophobic domain.[17–19] The 3-difluoromethylpyrazole carboxamide motif has proven the most broadly applicable pharmacophore, while the hydrophobic domain is represented by a great deal more structural diversity. Two notable examples are the norbornane-fused anilines found in benzovindiflupyr and isopyrazam.[17] These species possess unique three-dimensional structures and contain localized bulk near the pharmacophoric motif, two factors that communicated the potential for our 1-aminoNB substrates to serve as the basis of a new class of SDHI candidates.

To generate said SDHI candidates, the previously reported library of Schiff base-protected 1-aminoNBs were converted to the desired pyrazole carboxamides through a two-step solvolysis and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)-coupling sequence; a general protocol and the yields for the individual sequences can be found in the supplemental information. The library is represented in Figure 2, predominantly comprising the 3-difluoromethyl-pyrazole carboxamide. The compounds were evaluated using a poison plate mycelial growth assay, in which an active mycelial culture is positioned in the center of a plate containing medium inoculated with the compound of interest; radial growth is measured and then compared to growth on an untreated control (data reported as percent relative growth). The three pathogens chosen for this initial evaluation were *Fusariumgraminearum*, *Sclerotinia sclerotiorum*, and *Macrophomina phaseolina*, three agriculturally relevant fungal pathogens in the *Ascomycota* phylum for which there are currently a limited number of effective fungicides.

Gratifyingly, many of the 1-aminoNB carboxamides demonstrated fungicidal activity relative to untreated controls, at times matching the performance of the commercial fungicide fluxapyroxad. In general, *S. sclerotiorum* isolates were the most sensitive, with a few notable exceptions arising from *F. graminearum* and *M. phaseolina*; three isolates of each species were tested, although only five representative isolates are presented in Figure 2 for clarity (see Figure S1 and Table S1 for complete dataset).

Evaluation of 1-aminoNB-based fungicidal candidates

Certain structure-activity relationships can be inferred from the data presented below. Among the C7-dimethyl series (**1–10**), the *in vitro* performance proved to be most heavily influenced by the heteroarene-fused variations, while little influence was observed with substituents on the benzene ring. For instance, the C11-Me compound **2** was effectively indistinguishable from parent 1-aminoNB lead **1**. However, the pyridine within compound **3** proved to be the most ineffective motif evaluated, leading to no observable fungicidal activity. The thiophene-fused system **4** and electron-rich C8-OMe system **5** showed mixed effects. Both modifications appeared to diminish activity in *F. graminearum* isolate Ph-1 (relative growth: **4** = 80% and **5** = 79% versus **1** = 65%), although the introduction of the thiophene motif in analog **4** may be somewhat beneficial against *S. sclerotiorum* isolate

1980 and *M. phaseolina* isolate W25 (relative growth: **4** = 72%/81% versus **1** = 85%/89%). Electron-withdrawing groups on the benzene ring showed little effect, as seen in the C9-substituted series −H (**1**), $-CF_3$ (**6**), −Cl (**7**), −F (**8**), although the C9-CF_3 compound **6** did appear to be the least active of the group. A pair of positional variants led to little influence on activity; the C9-F (**8**) versus C10-F (**9**) comparison and the C9-OMe (**10**) versus C8-OMe (**5**) provided nearly identical performances, reinforcing the notion that these motifs would simply lie in a large hydrophobic pocket and not directly influence binding.

The most influential alterations in the 1-aminoNB candidate library were the manipulations of the bridging carbon in the norbornane (C7) (see Figure 3). In general, both the C7-*syn* and C7-*anti* isomers of mono-substituted systems (**11–20**) were more active than the C7-dimethyl congeners (**1**, **7–9**); the addition of halogenation at C9 or C10 again proved minimally effective (compare *syn*-C7-Pr analogs **13** and **14** as well as *anti*-C7-Pr analogs **18** and **19**). The C7-methylene species **21**, prepared via decarboxylation of a C7-CO_2H (see supplemental information), also demonstrated improved performance. Interestingly, these C7 manipulations offered examples of isolate-specific performance against certain fungal species. The C7-methylene candidate **21** demonstrated excellent performance against *F. graminearum* isolate Ph-1 (relative growth: 11%), but little to no activity against the other isolates 104B (relative growth: 107%) or 66B (relative growth: 94%; data not shown), mirroring the activity profile of commercial fungicide fluxapyroxad. Alternatively, the addition of C7 substitution (*syn*-, *anti*-, or *gem*-dimethyl) led to diminished activity against isolate Ph-1 while slightly improving activity against isolate 104B. The latter activity is not sufficient to compete with modern commercial fungicides, but the presence of this apparent size exclusion-based inversion of isolate selectivity may prove informative for future development programs. A similar C7-mediated isolate specificity was seen in the performance against *S. sclerotiorum*. While the *anti*-mono-substituted systems **16** and **20** were only modestly active across all of the isolates, the *syn* C7-Pr isomers **11** and **15** were more active against isolate 1980 than isolate 274, even matching the performance of fluxapyroxad (relative growth against *S. sclerotiorum* isolate 1980: **fluxa** = 11%, **11** = 17%, **16** = 74%). C7-methylene compound **21**, the least bulky of the series, reversed this profile, showing modest activity against *S. sclerotiorum* isolate 1980 (relative growth: 80%), yet good activity against *S. sclerotiorum* isolate 274 (relative growth: 47%). Further investigation of the connection between these substitution patterns and the genomic profile of the individual isolates will be explored in due course.

Lastly, a small series of alternative pyrazoles were prepared to assess the potential to improve the pharmacophore itself (see Figure 4). The C5′-Cl species was chosen as a potential covalent inhibitor and did appear to offer improved activity (e.g., relative growth against *M. phaseolina* isolate W25, C5′-Cl compounds **22**, **23**, **24** = 47%, 45%, 97%, respectively, versus C3′-difluoromethyl congeners **1**, **8**, **3** = 89%, 79%, 97%, respectively). Interestingly, the pyrazole ester, available from a bridgehead hydro-deamination of the corresponding 1-aminoNB, also offered a modest improvement in activity (relative growth against *F. graminearum* 104B and *M. phaseolina* isolate W25: **1** = 79%/89% versus **25** = 46%/44%). Whether or not these pyrazole variations would retain these benefits in a field

setting relative to the proven 3-difluoromethylpyrazole carboxamide remains to be seen. Additional variants can be found in the supplemental information.

Significantly, the fungicidal activity described above was not limited to *in vitro* performance. A subset of compounds was tested against *F. graminearum* isolate Ph-1 in a greenhouse setting, evaluating preventive activity in spring wheat (cv. Wheaton). SDHI candidates were applied as 250 ppm stock solutions in acetone 24 h before inoculation with the *F. graminearum* isolate Ph-1, evaluating performance relative to commercial SDHI fungicide pydiflumetofen. Due to variation in a greenhouse setting, there were no statistically significant differences, but major trends align with *in vitro* data (see Figure 5). The C7-methylene compound **21** proved active, preventing disease progression at a level similar to the commercial control. While less active, the C7-mono-substituted systems **11** and **16** demonstrated performance that aligned with the radial mycelial growth assay, with the *syn*-substituted species **11** proving more effective than the corresponding *anti*-substituted isomer **16**. These data suggest the functional predictive capabilites of the *in vitro* assay for this collection of SDHI candidates. More important, these data demonstrate that our 1-aminoNB-based leads do not suffer from any inherent or systematic pharmacokinetic barriers to *in planta* function, although the locale of fungicidal function cannot be determined from the data (residual activity versus distribution into the plant tissue). While additional replications and studies would be needed to fully characterize the fungicidal activity of these 1-aminoNB-based leads *in vivo*, the observed *in planta* performance indicates the potential for translational development.

Structurally unique 1-aminoNB building blocks have been implemented in the design, synthesis, and evaluation of novel agrochemical fungicide candidates. These building blocks were only made available through photochemical innovation, showcasing the ability of synthetic discoveries to have immediate interdisciplinary impact. Certain 1-aminoNB SDHI leads showcased fungicidal activity that competed with commercial fungicidal agents *in vitro*, while also revealing isolate-specific activity profiles that can be used to inform next-generation design. Importantly, the 1-aminoNB compounds also demonstrated *in planta* activity against the prominent fungal pathogen *Fusarium graminearum*, highlighting the potential for this class of compounds (and thus the photochemical methodology) to influence industrial agrochemical development.

EXPERIMENTAL PROCEDURES

Resource availability

Lead contact—The lead contact is Corey R.J. Stephenson (crjsteph@umich.edu).

Materials availability—Detailed procedures to generate these unique analogs can be found in the supplemental information. A generic protocol and photochemical reaction setup are depicted in Scheme S1 and Figure S4, respectively. NMR and other characterization data are provided, including the NMR spectra (see Figures S5–S88). The compounds reported herein may be obtained and used for research purposes under the auspices of a material transfer agreement and/or related confidentiality agreements with the

University of Michigan; requests and inquiries can be sent to Prof. Corey R.J. Stephenson (crjsteph@umich.edu).

Data and code availability—This study did not generate datasets or codes. Full experimental procedures and corresponding datasets are provided in the supplemental information (see Figures S1–S3 and Table S1).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

The authors acknowledge the financial support for this research from the NIH NIGMS (R01-GM127774) and the University of Michigan. D.S. was supported by a Postdoctoral Fellowship, PF-16-236-01-CDD, from the American Cancer Society. J.L.C. was supported by the University of Michigan's Rackham Merit Fellowship. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship (to R.C.M., grant no. DGE 1256260). This work was supported by the ADVANCE Grant Proof of Concept Fund, sponsored by the Michigan Economic Development Corportation (MEDC).

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Highlights

- Photochemical advances enable new design tactic in fungicidal development
- *In vitro* and *in planta* fungicidal activity found with 1-aminonorbornane scaffold

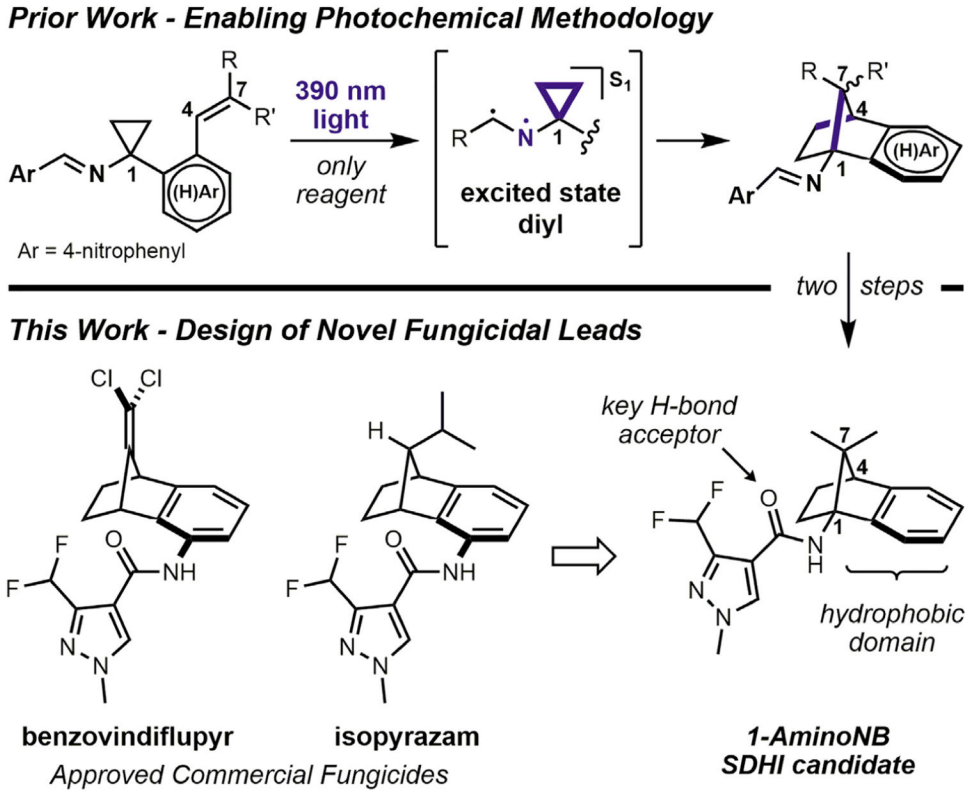

Figure 1. Design of novel fungicidal candidates as enabled by photochemical innovation

Generic synthetic approach to 1-aminonorbornanes is depicted, as is the basis for the design of the proposed SDHI candidate class.

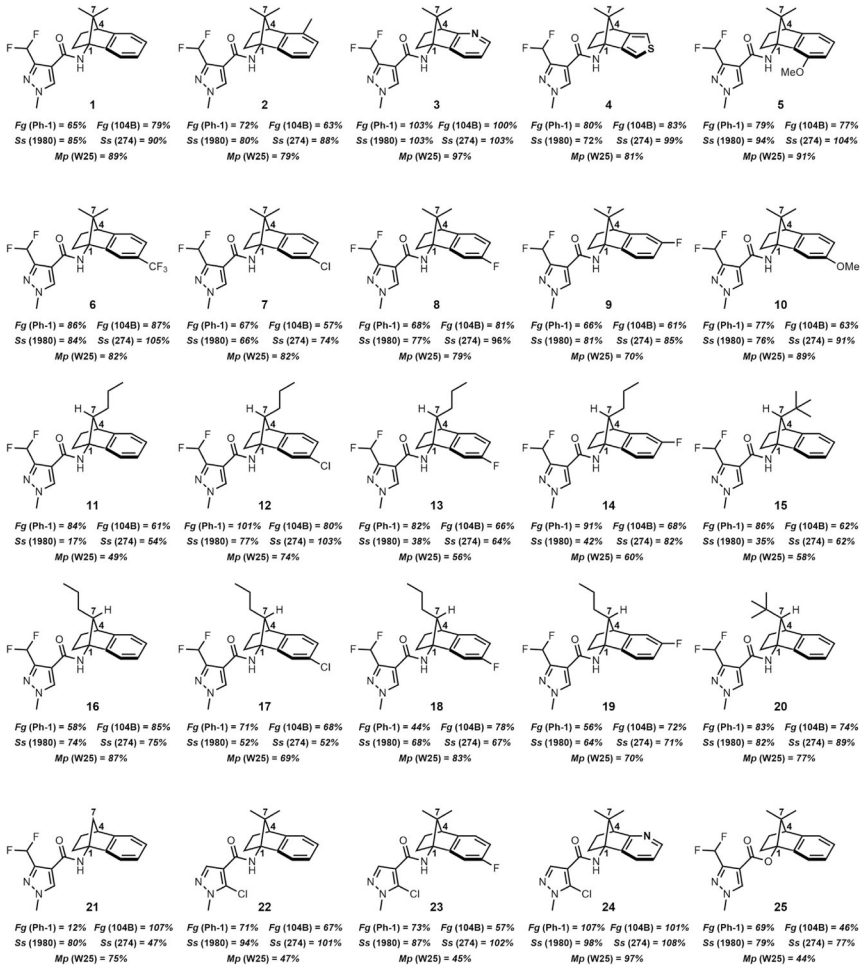

Figure 2. Fungicidal activity of 1-aminoNB SDHI candidates—percent radial mycelial growth

All values reported as the percent relative growth relative to an untreated control (untreated control set to 100% by convention; experimental details provided in the supplemental information); the fungi abbreviations are as follows: *Fg, Fusarium graminearum*; *Ss, Sclerotinia sclerotiorum*; *Mp, Macrophomina phaseolina*; the specific fungal isolate is provided in parentheses; the standard error for each value is provided in the supplemental information. Fluxapyroxad, a commercial SDHI (not shown), was used as a positive control; data provided in the supplemental information and reported in the text where applicable ("fluxa"). All compounds tested at 10 ppm concentration in agar growth medium (~30 μM for this class of compounds).

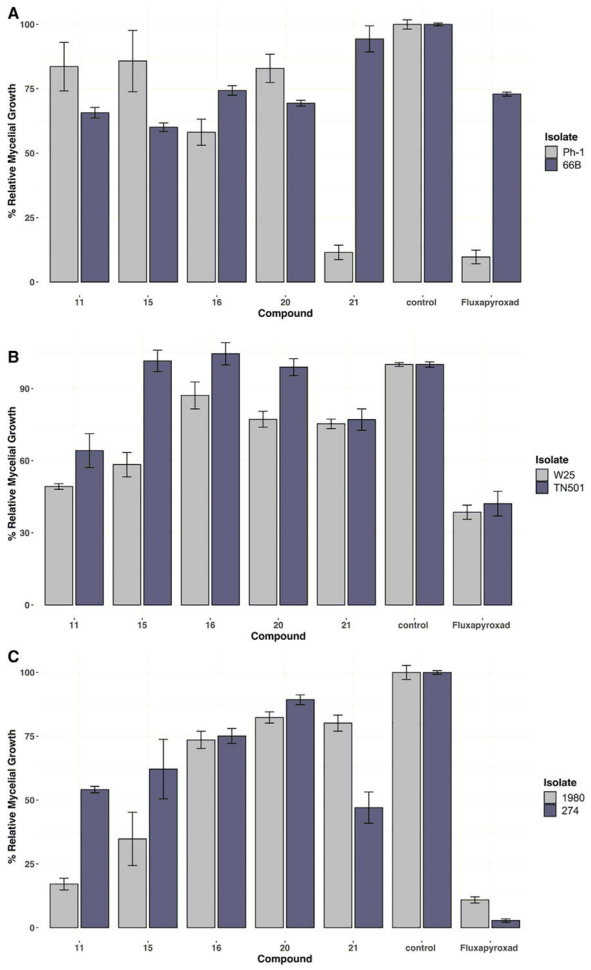

Figure 3. Isolate-specific fungicidal activity as a function of C7 substitution
See Figure 2 for description of assay and data representation.

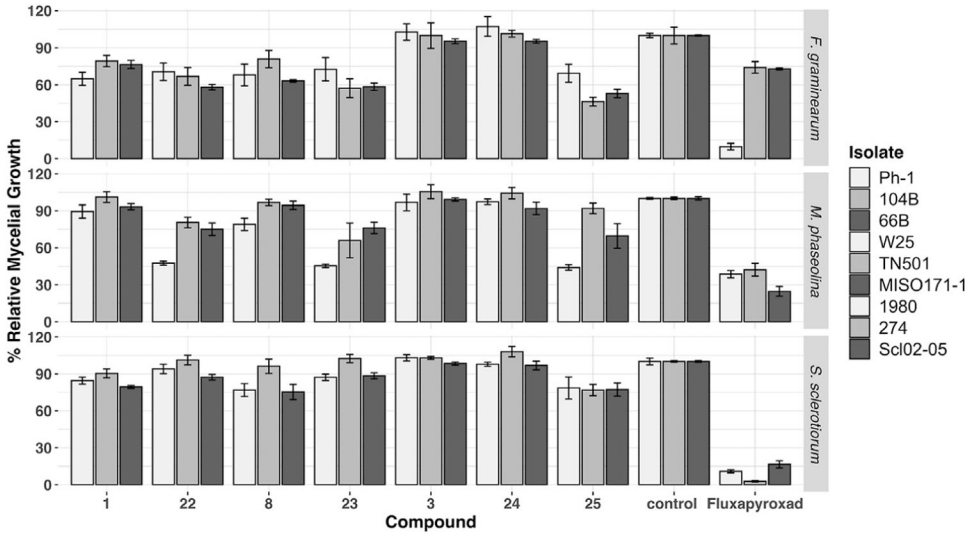

Figure 4. Comparison of analogs with alternative pyrazole motifs

See Figure 2 for description of assay and data representation.

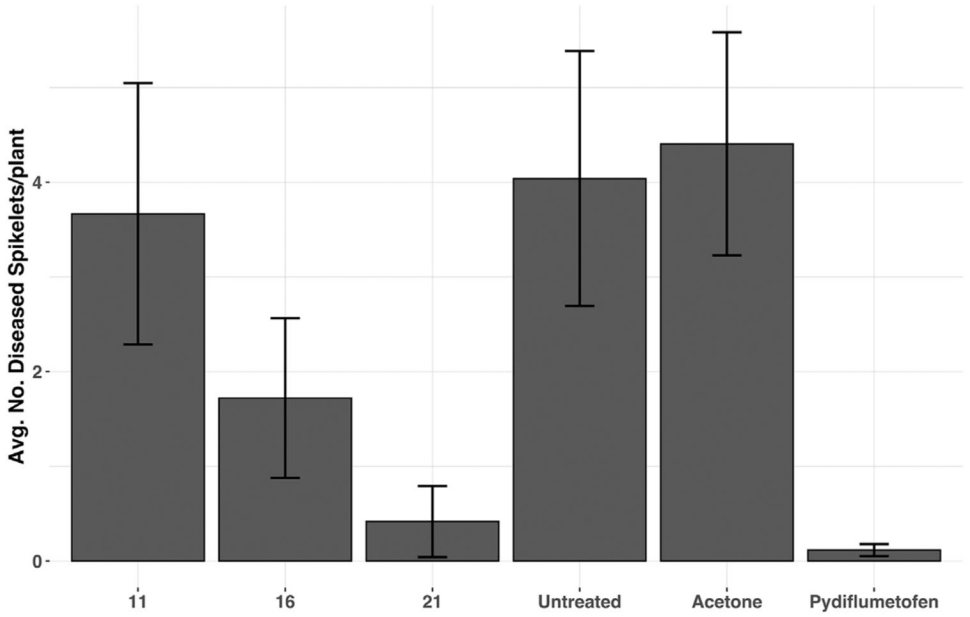

Figure 5. *In planta* performance of 1-aminoNBs

Results of *in planta* testing in a greenhouse setting, a 250-ppm solution in acetone (~750 μM for these compounds) was sprayed on wheat heads 24 h before spraying heads with *F. graminearum* conidia (isolate Ph-1) with surfactant tween at anthesis in a susceptible spring wheat variety. A maximum of 600 μL was applied to each head, leading to an estimated maximum dose of 0.15 mg per head. Approximately 21 days after inoculation, wheat heads were rated for necrosis (sign of infection) by counting the number of affected spikelets per head. Three pots with at least 3 plants per pot were used in each experiment, and 2 runs were completed, with the mean of both runs presented here, and error bars representing the standard error of the mean.