Total synthesis and structure-activity-relationship of Alternaric Acid delivers an herbicide vector

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Abstract: Global food security is one of the foremost challenges of our time requiring a multifaceted solution. Crop protection strategies are an essential part of this response; however, there is increasing resistance to known modes of action (MoA). Since its discovery in 1949, the natural product Alternaric acid has been proposed as a starting point for herbicide development. However, this target is undeveloped owing to its poor synthetic accessibility and a lack of knowledge of the associated pharmacology. Here we report the discovery of herbicidal compounds from Alternaric acid that operate via a potentially unknown MoA. Development of a total synthesis enabled structure-activity relationship (SAR) profiling of compound libraries, which, combined with phenotypic screening and molecular modelling data, identified small molecule lead compounds with enhanced and broader spectrum herbicidal activity than Alternaric acid.

Introduction

The global population is anticipated to increase substantially in the next few decades, reaching 9−10 billion by 2050.[1] This population expansion will place further demands on already polarized global food production. To meet these demands, effective crop protection strategies will become increasingly essential to maintain and improve yield from arable land; however, agrochemical effectiveness is being compromised by resistance.[3] Over 60% of current herbicidal agents involve MoAs that are already associated with serious resistance issues.[4] Combined with loss of arable land,[5] urgent innovation is needed in the development of crop protection agents to meet the needs of future food security.[2] Specifically, in order to bypass developing resistance, there is a critical requirement for new agrochemicals that operate via novel MoA.

The most prominent approaches for lead generation in agrochemical discovery are designed libraries based on a novel target hypothesis, scaffold hopping from competitor assets, and natural product-based leads.[6] De novo target ID can lead to a new MoA but carries significant risk and is both time-consuming and costly. Scaffold hopping builds upon a large web of biological knowledge, but target and MoA novelty is low. However, the molecular complexity and rich diversity of natural products offers an attractive strategy for the discovery of a novel MoA. This strategy has seen significant success with several important herbicides originating from natural phytotoxins,[7−10] such as the highly successful HPPD inhibitor Mesotrione (**1**, Fig. 1A).[7,11]

Alternaric acid (**2**, Fig. 1B), first isolated in 1949 by Brian and co-workers,[12] is a phytotoxin produced by the phytopathogenic fungus *Alternaria solani*, which is the causal fungus of early blight disease in potato and tomato crops.[13−15] Early biological assessment identified **2** as possessing herbicidal and fungicidal activity.[12−14] The first total synthesis and stereochemical determination of Alternaric acid was first achieved in 1994 by Ichihara and co-workers in 29 steps (0.001% yield), giving 16 mg of **2**.[16,17] The same year, Ichihara and co-workers reported that **2** exhibited phytotoxic activity against tomato seedlings.[18] However, the MoA remains unknown[19,20] and limited SAR has been reported: the only data relates to an observed loss of activity when the C10 methylene or C15 hydroxyl motifs are removed (Fig. 1B).[18,21] The principal issue preventing systematic analysis of **2** as a herbicidal lead has been the lack of synthetic access to the natural product as well as an approach to systematic editing of the structure to fully explore SAR.

Here, we report a practical, scalable synthesis of Alternaric acid (**2**) *via* a key intermediate from which crucial structural modifications can be achieved in only two steps. This has allowed extensive exploration of molecular space to establish SAR and enabled the design and synthesis of analogues. In turn, this has led to the

discovery of a new class of structurally less complex and more developable lead compounds that displays superior herbicidal activity and with a broader spectrum profile operating via an unidentified MoA (Fig. 1C).

Results and Discussion

Objectives. The primary objective of this synthetic campaign was to produce significant quantities of **2** to enable broad biological evaluation and establish meaningful SAR on this elusive target. This is an early-stage project with the key aim to establish viability of the Alternaric acid-mediated phenotypic response as a target for further development. A cursory analysis of **2** immediately highlights the triketone motif, which is reminiscent of similar warheads in other herbicides, such as **1**.[7] We therefore anticipated that this unit could significantly impact activity. Consequently, our strategy was to build **2** from the union of the 'head group' **4** with the larger carboxylic acid 'tail' component **3** (Fig. 2A). This would allow rational analysis of the impact of each fragment and their constituent functional groups.

Synthesis of Alternaric Acid. Our optimized synthetic route, built on previous work by Trost,[22] is described in Fig. 2B (see ESI pS7-S19 for full details). Beginning with commercially available alcohol **5**, one-pot Swern oxidation-Wittig olefination afforded the unsaturated ester **6** in 93% yield. A one-pot debromination-dehydrobromination then provided the vinyl bromide **7**, which was used in an sp^2-sp^3 Suzuki–Miyaura[23] coupling with alkyl borane **9** (accessed *via* hydroboration of **8**) to yield **10** in 96%. The use of Xantphos[24] as a ligand was found to be critical for the success of the coupling (see Table S1). Sharpless dihydroxylation[25] of **10** yielded the corresponding diol **11** in 93% yield as a single diasteroisomer. Diol **11** was temporarily protected as the acetonide in a one-pot procedure during TBDMS removal to give **12**, which underwent Grieco elimination[26] of the primary alcohol to deliver alkene **13.** Acetonide deprotection afforded the free diol **14**. All yields in this sequence were >90% per step. From **13**, our initial plan was for temporary diol protection as a carbonate at this stage, which would be removed during ester saponification in the last step in the route; however, the Grieco elimination failed in presence of the carbonate, and the free diol was also incompatible with the elimination, necessitating this protecting group switch.

Using **14**, we employed Trost's Alder-ene-type reaction[22,27,28] using catalytic $CpRu(MeCN)_3PF_6$ with alkyne **15**, which was prepared in one step from the corresponding commercially available carboxylic acid. This provided skipped diene **16** in 63% yield. Protection of diol **16** as the carbonate using triphosgene was accomplished in 92% yield and selective hydrolysis of the Fmoc ester gave key intermediate **18**.

Head group tricarbonyl **4** was prepared in two steps from commercially available enantiopure starting material and coupled with acid **18** in an esterification/Fries-type rearrangement sequence[29] to give compound **19**. Final hydrolysis of both the carbonate and the methyl ester successfully afforded Alternaric acid (**2**) in only 12 steps with 21% overall yield. The structure and absolute stereochemistry was unambiguously confirmed by obtaining and X-ray structure of this natural product.

Biological screening of Alternaric Acid. The herbicidal activity of Alternaric acid was first reported in 1949[12–14] but very limited progress has been made with this asset due to the difficulties in obtaining material. However, the above route enabled production of significant quantities of pure **2** that allowed evaluation of biological activity using phenotypic assays at 1000 g ha^{-1} against a range of weed species (Fig. 3). At the 1000 g ha^{-1} rate, **2** demonstrated good levels of herbicidal activity with almost complete control of the target for dicot weeds (*Amaranthus retroflexus* (AMARE) and *Stellaria media* (STEME)) both post- and pre-emergence. High level of phytotoxicity was also observed against the monocot weed DIGSA pre-emergence with necrosis and stunting symptomology dominant. Interestingly, despite the head group of **2** having similarities to mesotrione (**1**) and related herbicides, bleaching, a symptom characteristic of HPPD inhibition, was not observed. These observations were further supported by computational modelling using the *Arabidopsis* HPPD crystal structure model, which indicated low likelihood of activity *via* this signalling axis (Fig. 4). Control processes confirmed that tail group **3** and head group **4** were inactive, indicating that **2** was not acting as a pro-cide (a labile precursor to a biologically-active herbicide) and that both units were essential for phytotoxicity. Further assessment through a proprietary MoA phenotypic screening platform against a broad series of defined targets could not identify the mode of action of **2**. Collectively, these data suggested that **2** was potentially exhibiting its herbicidal effects via an unknown MoA.

Development of an herbicidal lead compound from Alternaric Acid. Despite promising activity and the potential for unknown MoA, an herbicidal solution is only useful if a tractable lead can be identified for development. The structural complexity of **2** is incompatible with the requirements for production on large scale, and at acceptable cost.[30] The synthetic strategy outlined above was therefore expanded into a bifurcated SAR and discovery campaign (Fig. 5A). The complexity of the tail structure **3** was the target of focused libraries designed to extract information about SAR chemical space. The purpose of this was to establish the essential contributors to potency and the minimum structural requirements to maintain activity without crossover to other signaling axes, such as HPPD. The selection of carboxylic acids was guided by pre-screening to align with modern concepts of agrichemical design, including application of standard physchem filters (molecular weight, lipophilicity) as well as commercial availability of the chosen fragments. The head group **4,** while lacking potency as described above, was simple enough for development if suitable modifications could be made to re-establish potency. Accordingly, **4** was the subject of structural elaboration via parallel synthesis to re-establishing activity via the new MoA, again avoiding target crossover. The combined 'bottom-up, top-down' approach was envisioned to allow the combination of datasets and enable the identification of developable lead series for this unknown MoA.

The libraries of compounds (see ESI pS20-S81 for full details) were again assessed for pre- and post-emergence phytotoxicity in phenotypic screens at 1000 g ha^{-1} against four weed species, with **2** and commercial herbicides used as controls for comparison. Illustrative excerpts from this analysis are provided in Fig. 5B.

All analogues showed higher levels of weed control when applied post-emergence. Inverting the stereochemistry of the methyl group had little impact (**20** vs. **2**), while deletion (**21**) or homologation (**22**) had a slightly negative impact. A more pronounced loss of activity was observed with ketone **23** and lactam **24** analogues of **21**. This suggests that the presence of the methyl substituent in the head group plays an important mechanistic role, with very little room for modification of this motif away from that found in the natural product.

Interestingly, the diversity screen revealed several compounds with attractive structural simplicity, which exhibited good phytotoxicity, despite being slightly less active than the head group series (see ESI pS181-S185 for full details). In general, compounds bearing a heterocycle (**41**, **42**, **43**, and **44**) showed good herbicidal activity, especially when applied post-emergence. Sulfonamide **47** also demonstrated high herbicidal activity. Significantly, the simple amide derivative **48** exhibited excellent phytotoxic activity in both pre- and post-emergence, demonstrating increased potency compared to the natural product progenitor **2** and very similar to the commercial standards included in the test. The summarized key learnings from this campaign were two-fold: (1) that the head group is essential for activity and editing of this is not tolerated and (2) the tail component can be significantly edited to generate considerably fewer complex analogues, which exhibit similar as greater activity as the natural product.

Based on these findings, we undertook a second-round amide focused library synthesis based on **48** (see Scheme S3). Overall, all compounds demonstrated good activity, especially post-emergence (Table S6). Several compounds in the amide variation subset (Fig. 6) were particularly promising. Specifically, cyclic amides such as the morpholine amide **55** (not shown, see pS62 and Tables S6 and S8), azetidine amide **59,** and indoline amide **62** reached >90% control of at least two weed species in these phenotypic screens; however, compound **48** remained the most active of all compounds assessed.

Compounds **48**, **59**, and **62** were selected for progression to higher tier profiling phenotypic screening with larger plants – the data are compared to Alternaric acid **2** (Fig. 6 and Table S7). In this assay, the phytotoxicity was assessed visually against six weed species at different rates. Compound **48** retained significant post-emergence activity even at 500 g ha^{-1} against both broad leaf and grass weeds, compared to the natural product starting point (**2**), which showed good levels of control only against *Amaranthus spp*. Additionally, compound **59** performed well in this assay with high phytotoxicity post- and pre-emergence and activity being maintained at 500 g ha^{-1}. Compounds **48** and **59** both resulted in bleaching and chlorosis symptoms when tested in the higher tier assay. Since bleaching is a characteristic symptom associated with HPPD inhibition, this observation prompted us to evaluate *in vitro* activity of these compounds against the HPPD plant enzyme

(Table S8); however, low binding affinity was observed in this assay for most of the synthetic analogues tested. This suggests that other factors could contribute to the observed biological efficacy, in addition to HPPD inhibition. Furthermore, compound **62**, which exhibited very encouraging weed control, especially at the 1000 g ha^{-1} rate (Fig. 6), did not induce bleaching, with stunting and necrosis observed instead, like the natural product **2** (Table S7). Compounds **48**, **59**, and **62** also displayed a lack of HPPD activity in biochemical *in vitro* assays against plant HPPD enzyme (Table S8). Collectively, these results led us to hypothesise that the amide derivatives **48**, **59**, and **62** may be operating *via* a different MoA or perhaps a combination of HPPD activity coupled to an unknown MoA, as observed for Alternaric acid (**2**). Binding to HPPD was again evaluated via modelling (Fig. 7), which suggested a poor interaction, offering support for the yet as unidentified MoA. Lastly, a preliminary comparative analysis of the data also indicated some potential crop injury to *Zea mays* (Fig. 6 and Table S7), which could potentially be a useful signal for development of a burndown concept.

In summary, an extensive synthetic study around the natural product and phytotoxin Alternaric Acid has been accomplished. The development of a robust 12-step gram-scale synthesis to produce quantities of the natural product allowed extensive biological profiling *in vivo*. This confirmed a narrow spectrum regarding biological efficacy, coupled with structural complexity owing to the presence of several polar groups that could impact bioavailability and *in planta* stability. Through the gram-scale synthesis of a key intermediate, the preparation of natural product derivatives was carried out efficiently, enabling SAR investigations of the head group moiety. With the methyl-substituted dihydro-pyran-dione identified as a key constituent for herbicidal activity, a range of analogues were designed and synthesized with a view to simplify the alkyl chain moiety of Alternaric acid whilst retaining good phytotoxicity. Gratifyingly, three new structurally simpler amide derivatives were found to exhibit excellent herbicidal properties and broader spectrum than the original natural product. These promising compounds represent a class of lead compounds for herbicidal discovery with an unknown mode of action related to Alternaric Acid. The very early-stage data invites further investigation of the molecular basis for phenotypic response before crop selectivity or specificity can be established.

Methods

General procedure for esterification/Fries-type rearrangement. A mixture of EDCI (1.1 equiv), the appropriate head group (1.1 equiv), DMAP (1.1 equiv), and the appropriate carboxylic acid (1.0 equiv), was dissolved in anhydrous MeCN or CH_2Cl_2 (0.20 M) and the resulting mixture was stirred at rt for 24 h. The reaction mixture was diluted with H_2O, acidified with 2 M aq. HCl and extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography (silica gel) to afford the desired product.

Compound 6. DMSO (9.70 mL, 136 mmol, 3.0 equiv) was added dropwise to a solution of $(COCl)_2$ (5.80 mL, 68.1 mmol, 1.5 equiv) in anhydrous CH_2Cl_2 (100 mL) at −78 ºC and the resulting mixture stirred for 30 min. A solution of (*S*)-2-methylbutan-1-ol (5) (4.90 mL, 45.4 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL) was added dropwise and the mixture stirred at −78 ºC for 1 h. Et_3N (31.6 mL, 227 mmol, 5.0 equiv) was added and the reaction mixture allowed to warm to rt and stir for 1.5 h. A solution of methyl (triphenylphosphoranylidene)acetate (15.2 g, 45.4 mmol, 1.0 equiv) in CH_2Cl_2 (40 mL) was added and the resulting mixture stirred at rt for 24 h. The reaction mixture was acidified with 10% aq. HCl and extracted with CH_2Cl_2. The organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography (silica gel, 5% Et_2O in petroleum ether) to afford the desired product as a colorless oil (5.99 g, 93%).

Compound 7. Bromine (86 µL, 0.70 mmol, 1.0 equiv) was added dropwise to a solution of **6** (100 mg, 0.70 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (4.7 mL) at 0 ºC and stirred. The mixture was allowed to warm to room temperature. After 2 h, the mixture was cooled to 0 ºC and Et_3N (0.49 mL, 3.52 mmol, 5.0 equiv) was added. The resulting mixture was stirred at rt for 14 h. The heterogeneous mixture was concentrated under reduced pressure. The crude residue was purified by column chromatography (silica gel, 5% Et_2O in petroleum ether) to afford the desired product as a colorless oil (112 mg, 72%).

Compound 10. To a solution of 9-BBN (2.0 M in THF, 88.4 mL, 44.2 mmol, 2.0 equiv) in anhydrous THF at 0 ºC was added (allyloxy)(*tert*-butyl)dimethylsilane (9.66 mL, 44.2 mmol, 2.0 equiv) and the mixture was stirred at rt. After 2 h, H_2O (2.0 mL, 111 mmol, 5.0 equiv) was added and the mixture transferred to a flask containing

a solution of vinyl bromide **7** (4.89 g, 22.1 mmol, 1.0 eq), $Pd(OAc)_2$ (247 mg, 1.10 mmol, 0.05 equiv), Xantphos (1.27 g, 2.20 mmol, 0.1 equiv), and K_3PO_4 (14.1 g, 66.3 mmol, 3.0 equiv) in THF (100 mL). The resulting mixture was heated to reflux for 14 h. The mixture was allowed to cool to rt then diluted with H_2O and extracted with Et_2O. The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography (silica gel, 0–5% Et_2O in petroleum ether) to afford the desired product as a colorless oil (6.67 g, 96%).

Compound 11. A mixture of potassium carbonate (8.76 g, 63.4 mmol, 3.0 equiv), potassium ferricyanide (20.9 g, 63.4 mmol, 3.0 equiv), $(DHQD)_2PHAL$ (823 mg, 1.06 mmol, 0.05 equiv), osmium tetroxide (5.60 mL, 0.85 mmol, 0.04 equiv) in H_2O, methanesulfonamide (2.11 g, 21.1 mmol, 1.0 equiv), and compound **10** (6.65 g, 21.1 mmol, 1.0 equiv) in ^{t}BuOH/H_2O (1:1, 200 mL, 0.11 M) was stirred for 24 h at 0 °C. The mixture was diluted with sat. aq. sodium dithionite and stirred until the mixture became homogeneous, then extracted with Et_2O. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography (silica gel, 40% Et_2O in petroleum ether) to afford the desired product as a colorless oil (6.69 g, 93%).

Compound 12. CSA (173 mg, 0.75 mmol, 0.2 equiv) was added to a solution of compound **11** (1.30 g, 3.73 mmol, 1.0 equiv) and 2,2-dimethoxypropane (4.6 mL, 37.3 mmol, 10 equiv) in acetone (20 mL) at rt. The mixture was stirred for 24 h at rt before cooling to 0 ºC and addition of pyridine hydrofluoride (1.9 mL, 22.4 mmol, 6.0 equiv) and stirring for 1 h. The mixture was diluted with H_2O and extracted with Et_2O. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography (silica gel, 20–50% Et_2O in petroleum ether) to afford the desired product as a colorless oil (919 mg, 90%).

Compound 13. Tri-*n*-butylphosphine (2.84 mL, 11.4 mmol, 2.0 equiv) was added to a solution of compound **12** (1.56 g, 5.69 mmol, 1.0 equiv) and *o*-nitrophenylselenocyante (2.58 g, 11.4 mmol, 2.0 equiv) in anhydrous THF (30 mL). The mixture was stirred for 12 h at rt before addition of $NaHCO_3$ (955 mg, 11.4 mmol, 2.0 equiv) followed by the addition of H_2O_2 in H_2O (30% w/w, 5.91 mL, 56.9 mmol, 10 equiv). The mixture was stirred for 2 h then treated with 10% aq. HCl and extracted with Et_2O. The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography (silica gel, 0–10% Et_2O in petroleum ether) to afford the desired product as a colorless oil (1.32 g, 91%).

Compound 14. Compound **13** (1.32 g, 5.15 mmol, 1.0 equiv) was dissolved in anhydrous CH_2Cl_2 (15 mL), TFA (8.0 mL), and H_2O (0.6 mL) at rt. The mixture was stirred for 12 h at rt then concentrated under reduced pressure. The crude residue was purified by column chromatography (silica gel, 25% Et_2O in petroleum ether) to afford the desired product as a colorless oil (1.09 g, 99%).

Compound 15. A solution of 4-pentynoic acid (1.13 g, 11.5 mmol, 1.0 equiv), 9-fluorenylmethanol (2.48 g, 12.7 mmol, 1.1 equiv), DCC (3.56 g, 17.3 mmol, 1.5 equiv), and DMAP (141 mg, 1.15 mmol, 0.1 equiv) in anhydrous CH_2Cl_2 (50 mL) was stirred at rt for 15 h. The mixture was filtered then concentrated under reduced pressure. The crude residue was purified by column chromatography (silica gel, 10% Et_2O in petroleum ether) to afford the desired product as a beige solid (2.64 g, 83%).

Compound 16. Compounds **14** (500 mg, 2.33 mmol, 1.0 equiv), **15** (773 mg, 2.80 mmol, 1.2 equiv) and $CpRu(MeCN)_3PF_6$ (101 mg, 0.23 mmol, 0.1 equiv were dissolved in anhydrous MeOH (8.0 mL) and the mixture stirred at rt for 12 h. The mixture was filtered through celite then concentrated under reduced pressure. The crude residue was purified by column chromatography (silica gel, 30–50% Et_2O in petroleum ether) to afford the desired product as a colorless oil (724 mg, 63%).

Compound 17. A solution of triphosgene (795 mg, 2.68 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (3.0 mL) was added to a solution of compound **16** (1.32 g, 2.68 mmol, 1.0 equiv) and pyridine (1.3 mL, 16.1 mmol, 6.0 equiv) in anhydrous CH_2Cl_2 (10 mL) at −78 ºC. The resulting mixture was stirred for 3 h at 0 ºC then 2 h at rt. The mixture was quenched with aqueous NH_4Cl and extracted with CH_2Cl_2. The organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by column

chromatography (silica gel, 25% Et_2O in petroleum ether) to afford the desired product as a yellow oil (1.38 g, 99%).

Compound 18. DBU (0.14 mL, 0.94 mmol, 1.1 equiv) was added to a solution of compound **17** (443 mg, 0.85 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (13 mL) and the mixture stirred at rt for 3 h. The mixture was diluted with H_2O, acidified with 1 M aqueous HCl, and extracted with CH_2Cl_2. The organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography (silica gel, 20% EtOAc in CH_2Cl_2) to afford the desired product as a yellow oil (282 mg, 97%).

Compound 19. A mixture of EDCI (135 mg, 0.71 mmol, 1.1 equiv), compound **4** (88.9 mg, 0.71 mmol, 1.1 equiv), DMAP (1.1 equiv), and compound **18** (200 mg, 0.59 mmol, 1.0 equiv), was dissolved in anhydrous CH_2Cl_2 (4.0 mL) and the resulting mixture was stirred at rt for 48 h. The reaction mixture was diluted with H_2O, acidified with 2 M aqueous HCl, and extracted with CH_2Cl_2. The organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography (silica gel, 10–20% EtOAc in CH_2Cl_2) to afford the desired product as a yellow oil (210 mg, 79%).

Compound 4. To a solution of freshly distilled diisopropylamine (2.08 mL, 14.8 mmol, 3.5 equiv) in anhydrous THF at 0 ºC was added dropwise ^{n}BuLi (2.5 M in hexane, 5.92 mL, 14.8 mmol, 3.5 equiv). The resulting LDA solution was stirred at 0 ºC for 20 min. The mixture was cooled to −78 ºC and ^{t}BuOAc (1.70 mL, 12.7 mmol, 3.0 eq) was added dropwise. The resulting mixture stirred for 40 min at −78 ºC. A solution of methyl (*R*)-3-hydroxybutanoate (500 mg, 4.23 mmol, 1.0 equiv) in anhydrous THF (20 mL) was added dropwise to the mixture at −78 ºC. The reaction was allowed to warm to −50 ºC and stirred for 2 h then allowed to warm to −15 ºC and stirred for 1 h. The reaction mixture was slowly quenched with H_2O, acidified with 1 M aqueous HCl and extracted with Et_2O. The organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was diluted with anhydrous CH_2Cl_2 (20 mL) and the solution cooled to 0 ºC. TFA (0.33 mL, 4.23 mmol, 1.1 equiv) was added dropwise, the mixture was allowed to warm to rt and was stirred for 24 h. The mixture was concentrated under reduced pressure and the crude residue was purified by column chromatography (silica gel, 0–5% MeOH in CH_2Cl_2) to afford the desired product as a beige solid (472 mg, 89%).

Compound 2. Compound **19** (30 mg, 0.067 mmol, 1.0 equiv) was dissolved in 2 M aq. LiOH/MeOH/THF (1:1:2, 2 mL) and the mixture stirred at rt for 15 min. The mixture was neutralized with 1 M aq. HCl and the organic solvents were removed under reduced pressure. The resulting mixture was extracted with CH_2Cl_2. The organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The desired product was obtained without further purification as a white solid (25.0 mg, 91%).

Data Availability Statement

All data generated during this study are included in this published article (and its supplementary information files). Analytical data generated during the current study are also available in the University of St Andrews repository, https://doi.org/10.17630/c41bcf9c-57cc-46f2-94cf-c9f7559554c5. Crystallographic data for compound **2** is available from the Cambridge Crystallographic Data Centre (CCDC) under Deposition Number 2169366.

Acknowledgements

E.M.I and A.J.B.W. thank Syngenta and EPSRC for an iCASE PhD studentship. We thank Dr Chris Martin and Russell Ellis at the Syngenta Jealott's Hill Chemistry Automation Platform for their support in reaction optimization and library purification.

Author contributions Statement

E.M.I. conceived and conducted the synthetic chemistry. J.C.-B. guided compound design and coordinated the biological screening. A.M.Z.S performed and analysed X-ray crystallography. E.M.I, J.C-B., and A.J.B.W. wrote the manuscript. A.J.B.W. conceived the chemistry and directed the project.

Competing Interests Statement

The authors declare no competing interests.

Figures

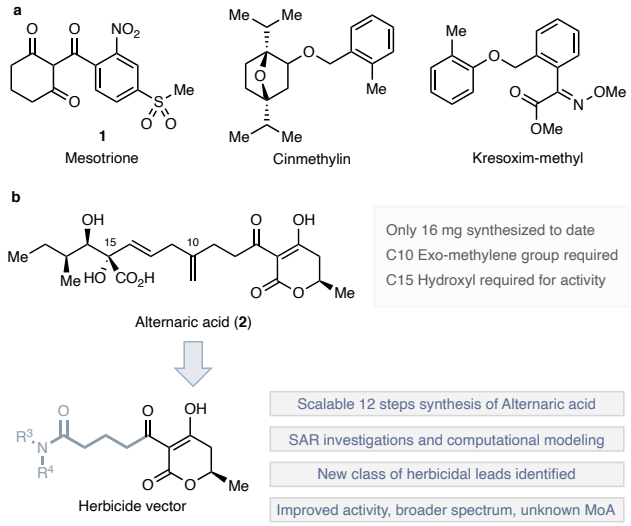

Figure 1. The use of natural product synthesis as a starting point for herbicide discovery. A. Exemplar marketed herbicides that were identified from investigation of natural products. **B.** Previous work on the total synthesis and SAR knowledge of Alternaric acid. **C.** This work: A combination of total synthesis, SAR profiling, computation, and biological screening delivers an herbicide vector from Alternaric Acid.

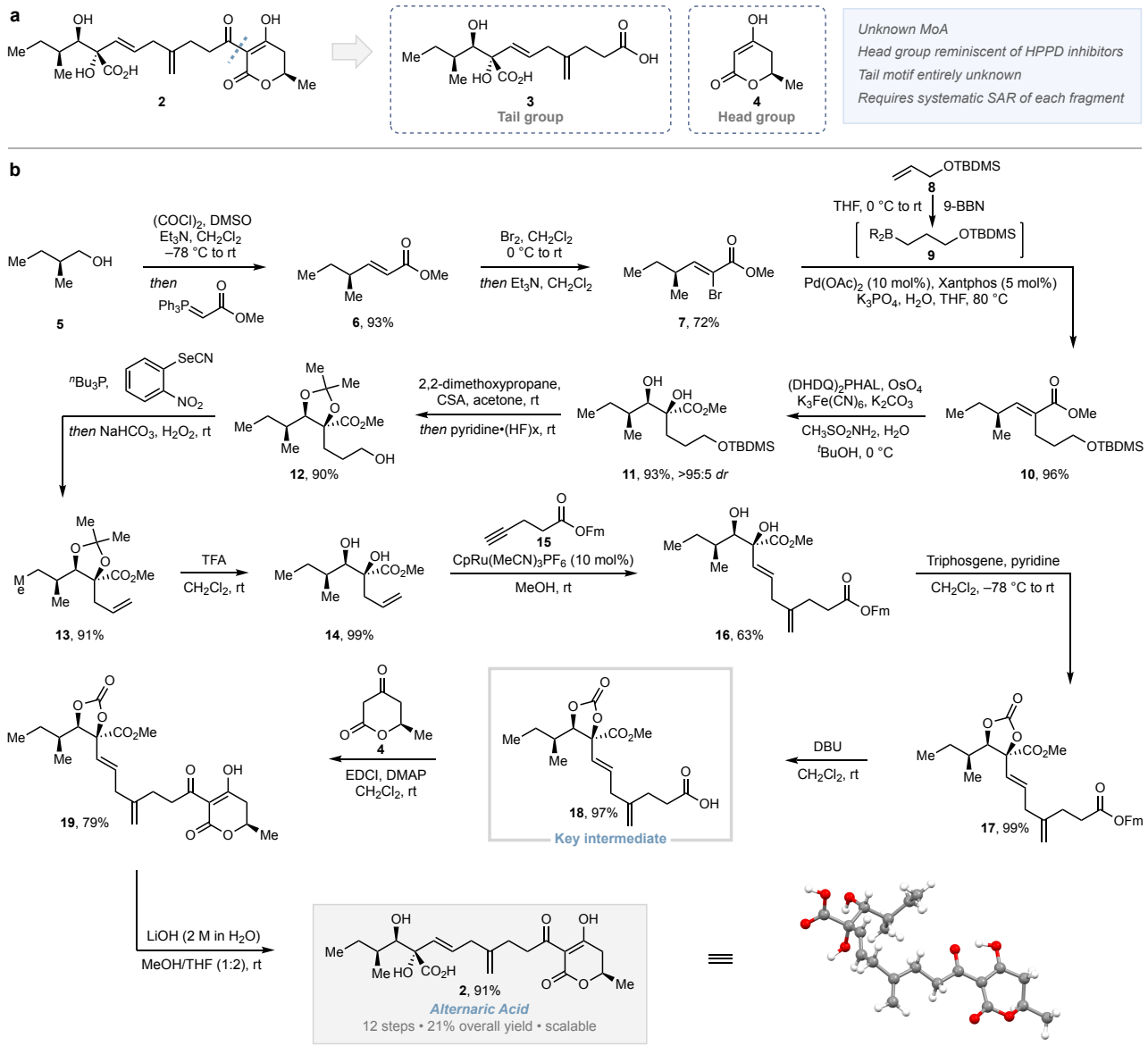

Figure 2. Synthetic strategy and total synthesis of Alternaric Acid. A. The main disconnections underpinning the synthetic strategy indicating the main components for SAR analysis. **B.** Practical and scalable synthesis of Alternaric acid (**2**). Transformations from compounds **5**-**18** performed on gram scale. 9-BBN, 9-borabicyclo[3.3.1]nonane; Cp, cyclopentadienyl; CSA, camphorsulfonic acid; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DMAP, 4-dimethylaminopyridine; $(DHQD)_2PHAL$, hydroquinidine 1,4-phthalazinediyl diether; DMSO, dimethylsulfoxide; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; rt, room temperature; TBDMS, *tert*-butyldimethytsilyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

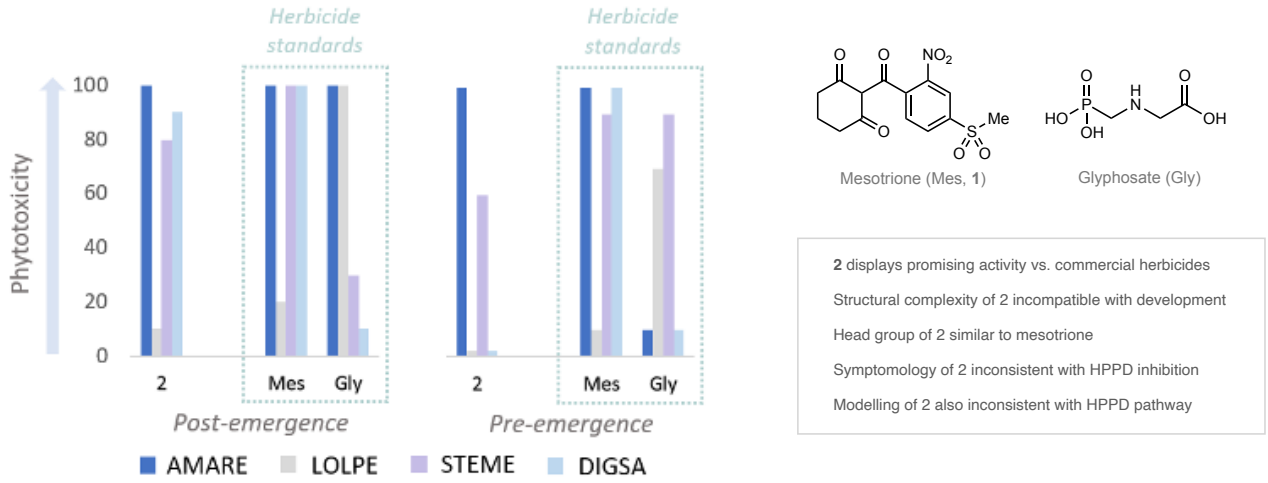

Figure 3. Biological screening of Alternaric Acid. Phytotoxicity assessment of **2** compared with commercial herbicides. In initial glasshouse screening (GH1), compounds are tested for pre- and post-emergence activity against four weed species at 1000 g ha^{-1}. Phytotoxicity is assessed visually (0-100%) where 100 is complete control of the target. Negative controls were untreated checks where no phytotoxicity (0%) was observed. Test species: *Amaranthus retroflexus* (AMARE), *Lolium perenne* (LOLPE), *Stellaria media* (STEME), *Digitaria sanguinalis* (DIGSA). Mesotrione (Mes, **1**) and Glyphosate (Gly) were used as positive controls.

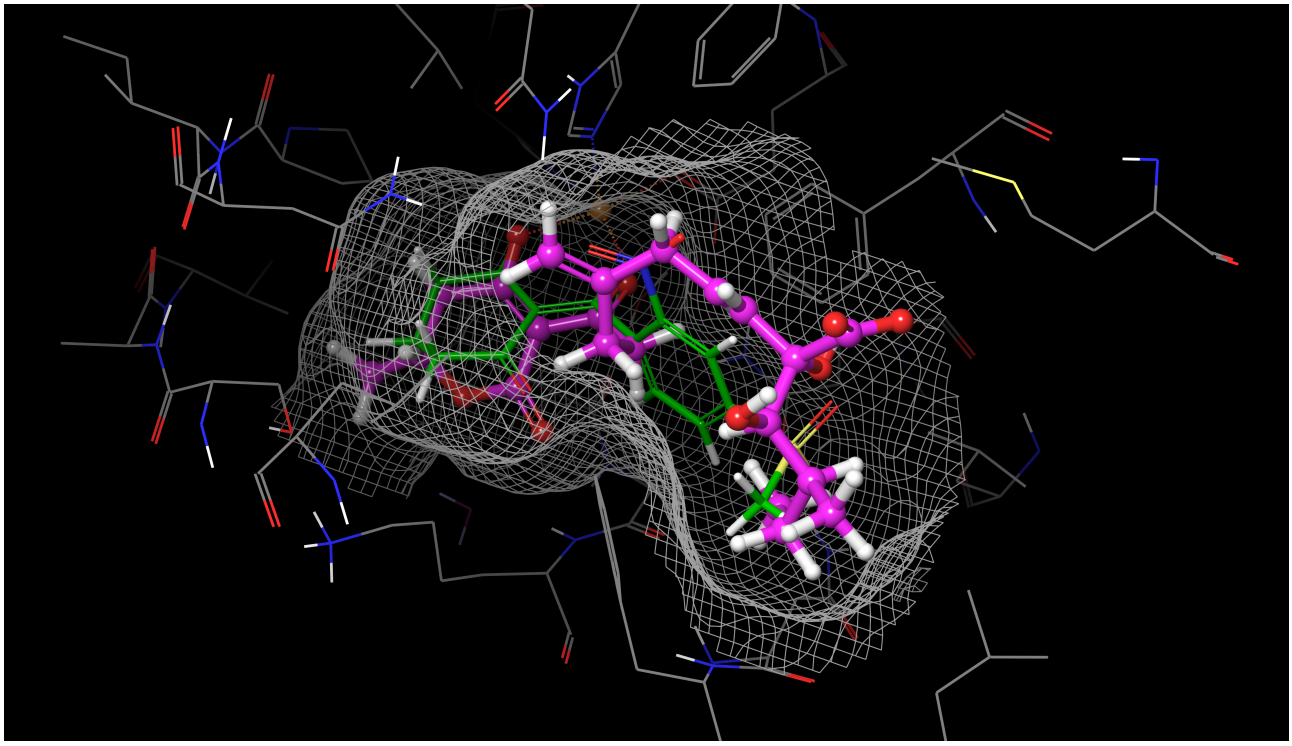

Figure 4. Computational model of HPPD binding of 2. Computational modelling of **1** (green) and **2** (pink) in *Arabidopsis* HPPD model indicating **2** has poor binding suggesting mode of action is not via HPPD, which is consistent with the symptomology.

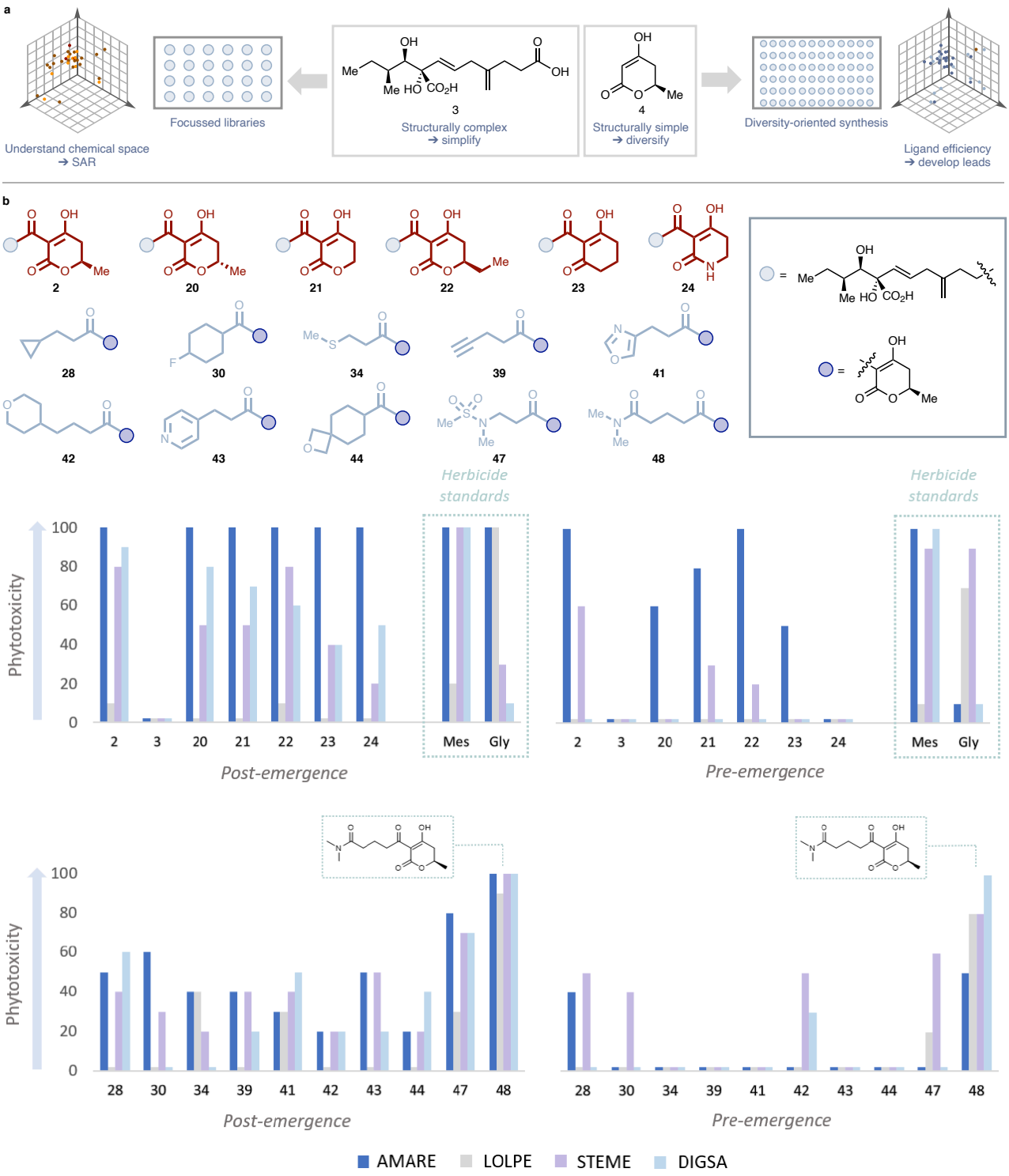

Figure 5. Lead development strategy and biological screening data of selected examples from the lead library. A. Bifurcated "bottom-up, top-down" approach to lead generation. **B.** Phytotoxicity evaluation of selected analogues. Compounds are tested for pre- and post-emergence activity against four weed species at 1000 g ha^{-1}. Negative controls were untreated checks where no phytotoxicity (0%) was observed.

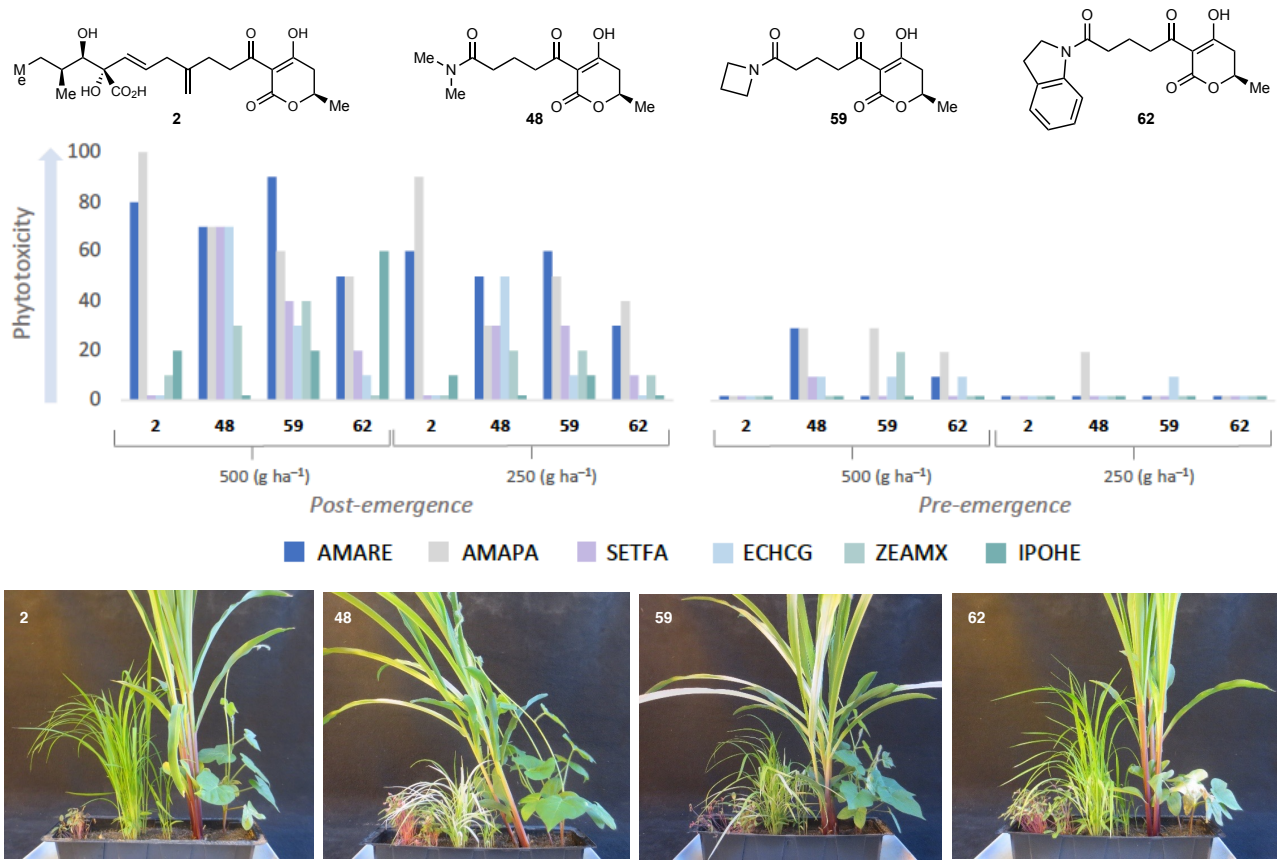

Figure 6. Advanced biological screening data of compounds from lead series. Phytotoxic evaluation of dimethylamide analogous compounds. In early profiling screen (EPS), compounds are tested for pre- and post-emergence activity against six weed species, with the compound applied at different rates (250 and 500 g ha^{-1}). Phytotoxicity is assessed visually (0-100%) where 100 is complete control of the target. Negative controls were untreated checks where no phytotoxicity (0%) was observed. Test species: *Amaranthus retroflexus (AMARE), Amaranthus palmeri (AMAPA), Setaria faberi (SETFA), Echinochloa crus-galli (ECHCG), Zea mays (ZEAMX), Ipomoea hederacea (IPOHE)*.

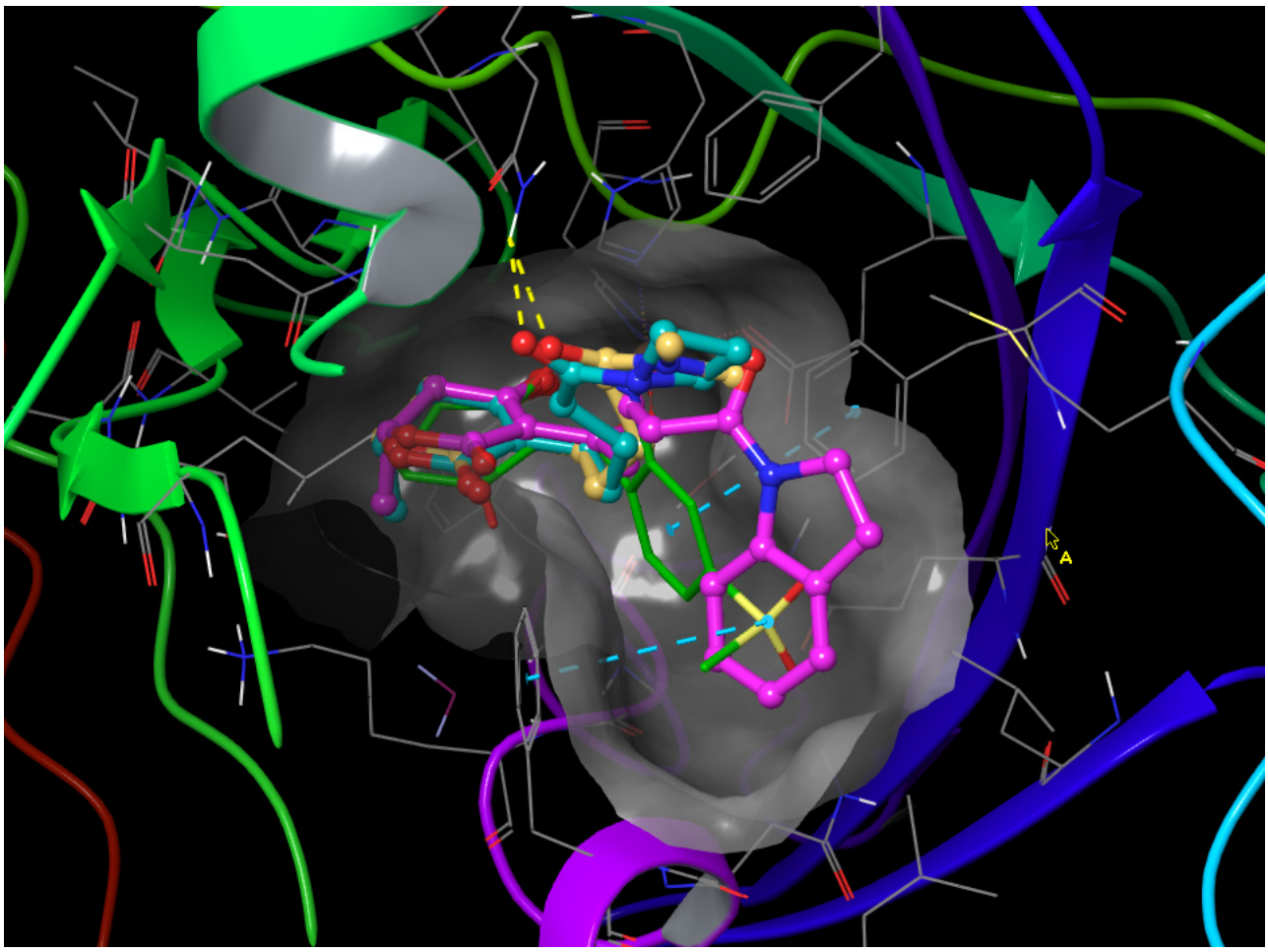

Figure 7. Computational model of HPPD binding of lead series. Computational modelling overlay of **1** (green), **59** (blue), and **62** (pink) in *Arabidopsis* HPPD model. HPPD inhibitors typically display and require aryl pharmacophore for activity. Lead compounds **59** and **62** lack this motif leading to ineffective binding in HPPD model, aligning with a lack of HPPD symptomology.

References

[1] Godfray, H. C. J. et al. Food security: The challenge of feeding 9 billion people. *Science* **327**, 812–818 (2010).

[2] Lamberth, C., Jeanmart, S., Luksch, T., Plant, A. Current challenges and trends in the discovery of agrochemicals. *Science* **2013**, *341*, 742–746 (2013).

[3] Hawkins, N. J., Bass, C., Dixon, A., Neve, P. The evolutionary origins of pesticide resistance. *Biol. Rev.* **94**, 135–155 (2019).

[4] Jeschke, P. Progress of modern agricultural chemistry and future prospects. *Pest Manag. Sci.* **72**, 433–455 (2016).

[5] Fitton, N. et al. The vulnerabilities of agricultural land and food production to future water scarcity. *Glob. Environ. Change* **58**, 101944 (2019).

[6] Loso, M. R. et al. Lead generation in crop protection research: a portfolio approach to agrochemical discovery. *Pest Manag. Sci.* **73**, 678–685 (2017).

[7] Mitchell, G. et al. Mesotrione: a new selective herbicide for use in maize. *Pest Manag. Sci.* **57**, 120–128 (2001).

[8] Grayson, B. T. et al. The physical and chemical properties of the herbicide cinmethylin. *Pestic. Sci.* **21**, 143–153 (1987).

[9] Sauter, H., Steglich, W., Anke, T. Strobilurins: Evolution of a new class of active substances. *Angew. Chem. Int. Ed.* **38**, 1328–1349 (1999).

[10] Bartlett, D. W. et al. The strobilurin fungicides. *Pest Manag. Sci.* **58**, 649–662 (2002).

[11] Loiseleur, O. Natural products in the discovery of agrochemicals *Chim. Int. J. Chem.* **71**, 810–822 (2017).

[12] Curtis, P. J. et al. Synthesis of cinerone, cinerolone and of cinerin-I. *Nature* **164**, 534–534 (1949).

[13] Brian, P. W. et al. Alternaric Acid; a biologically active metabolic product of *Alternaria solani* (Ell. & Mart.) Jones & Grout; its Production, Isolation and Antifungal Properties. *J. Gen. Microbiol.* 5, 619–632 (1951).

[14] Elson, G. W., Hemming, H. G., Wright, J. M., Brian, P. W. The phytotoxic properties of Alternaric Acid in relation to the etiology of plant diseases caused by *Alternaria solani* (Ell. & Mart.) Jones & Grout. *Ann. Appl. Biol.* ***39***, 308–321 (1952).

[15] Shahbazi, H., Aminian, H., Sahebani, N., Halterman, D. Effect of *Alternaria solani* exudates on resistant and susceptible potato cultivars from two different pathogen isolates. *Plant Pathol. J.* ***27***, 14–19 (2011).

[16] Tabuchi, H. et al. Stereochemistry of alternaric acid synthesis of the c9 c14 fragment. *J. Org. Chem.* ***59***, 4749–4759 (1994).

[17] Tabuchi, H. et al. Total synthesis of alternaric acid. *Tetrahedron Lett.* ***34***, 2327–2330 (1993).

[18] Tabuchi, H., Ichihara, A. Structures and stereochemistries of new compounds related to Alternaric Acid. *J. Chem. Soc., Perkin Trans. 1,* 125–133 (1994).

[19] Furuichi, N., Nishimura, S., Langsdorf, G. Effect of Alternaric Acid, a toxin of *Alternaria solani*, on the hypersensitive response of potato to *Phytophthora infestans*. *Ann. Phytopath. Soc. Japan* **58**, 1–7 (1992).

[20] Hassan, A. et al. Alternaric acid stimulates phosphorylation of His-tagged RiCDPK2, a calcium-dependent protein kinase in potato plants. *Genet. Mol. Res.* **11**, 2381–2389 (2012).

[21] Ichihara, A. Bioorganic chemistry of Alternaric Acid: Stereochemistry, total synthesis and bioactivity. *J. Synth. Org. Chem. Jpn.* **53**, 975–986 (1995).

[22] Probst, G. D., Schoop, A., Trost, B. M. Ruthenium-catalyzed Alder Ene type reactions. A formal synthesis of Alternaric Acid. *J. Am. Chem. Soc.* **120**, 9228–9236 (1988).

[23] Chemler, S. R., Trauner, D., Danishefsky, S. J. The B-alkyl Suzuki–Miyaura cross-coupling reaction: Development, mechanistic study, and applications in natural product synthesis. *Angew. Chem. Int. Ed.* **40**, 4544–4568 (2001).

[24] Kamer, P. C. J., Van Leeuwen, P. W. N. M., Reek, J. N. H. Wide bite angle diphosphines: Xantphos ligands in transition metal complexes and catalysis. *Acc. Chem. Res.* **34**, 895–904 (2001).

[25] Kolb, H. C., Vannieuwenhze, M. S., Sharpless, K. B. Catalytic asymmetric dihydroxylation. *Chem. Rev.* **94***,* 2483–2547 (1994).

[26] Gilman, S., Nishizawa, M., Grieco, P. A. Organoselenium chemistry. A facile one-step synthesis of alkyl aryl selenides from alcohols. *J. Org. Chem.* **41**, 1485–1486 (1976).

[27] Indolese, A. F., Mueller, T. J. J., Treptow, B., Trost, B. M. A Ru catalyzed addition of alkenes to alkynes. *J. Am. Chem. Soc.* **117**, 615–623 (1995).

[28] Machacek, M., Schnaderbeck, M. J., Trost, B. M. A Regioselective Ru-Catalyzed Alkene−Alkyne Coupling. *Org. Lett.* **2**, 1761–1764 (2000).

[29] Tabuchi, H., Hamamoto, T., Ichihara, A. Modification of the Fries type rearrangement of the O-enol acyl group using *N*,*N*-dicyclohexyl-carbodiimide and 4-dimethylaminopyridine. *Synlett* **1993**, 651–652 (1993).

[30] Duke, S. O., Dayan, F. E., Romagni, J. G., Rimando, A. M. Natural products as sources of herbicides: current status and future trends. *Weed Res.* **2000**, 99–111 (2000).