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A NEW POTENT INHIBITOR OF FUNGAL MELANIN BIOSYNTHESIS IDENTIFIED THROUGH COMBINATORIAL CHEMISTRY

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Abstract: A new fungicide lead has been identified by in vitro screening of a focused combinatorial library. Amides (768) were synthesized in pools of four and assayed as inhibitors of scytalone dehydratase. Deconvolution of one of the most active pools led to the discovery of a potent inhibitor of the enzyme **3b** (K_i = 26 pM), which has fungicidal properties.

Initiation of blast disease in rice plants by *Magnaporthe grisea* requires the fungal pathogen to melanize an infection structure[3] thus making the enzymes of the fungal melanin biosynthetic pathway in *M. grisea* highly attractive biochemical targets for the design of specific inhibitors for preventing the economically-significant disease. The fungal melanin biosynthesis pathway includes a series of successive reductions and dehydrations of 1,3,6,8-tetrahydroxynaphthalene to yield 1,8-dihydroxynapthalene, the last identified precursor of fungal melanin.[4] A naphthol reductase in the pathway is the target of several rice blasticides.[5] Scytalone dehydratase (SD) catalyzes the dehydrations of scytalone and vermelone in the pathway.[6] Carpropamid, a strong inhibitor of SD catalytic activity and competitive with respect to substrate,[7] has recently (1998) been commercialized as an agricultural fungicide useful for the control of rice blast.[8] Two additional inhibitors of SD, diclocymet [9] and AC 382042,[10] have been announced as rice fungicides under development. X-ray crystal structures of SD complexed with active-site inhibitors[7,11] have been exploited through structure-based design programs to yield potent inhibitors of the enzyme,[12] some of which have exhibited excellent disease control

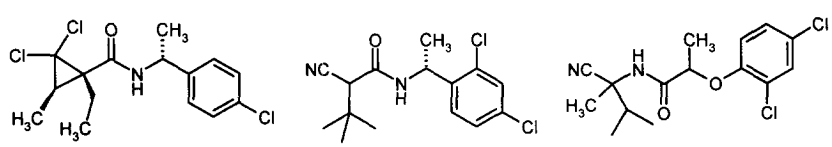

Figure 1. SD Inhibitors commercialized or under development as blasticides.

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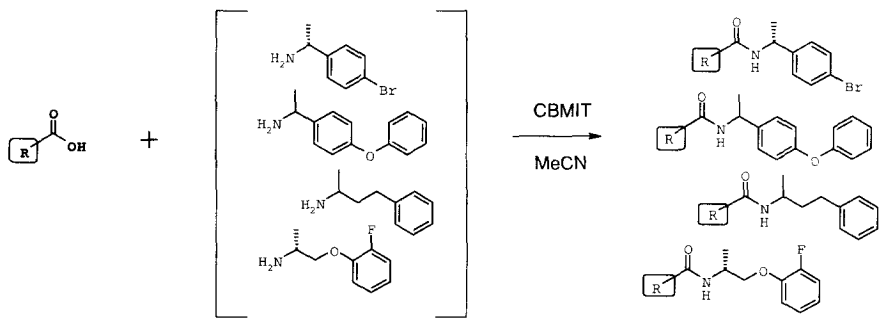
properties in evaluations of rice plants in the greenhouse and under cropping conditions. Unlike the reductase target in the pathway, which belongs to a large family of enzymes (short chain dehydrogenases),[13] SD has no known functional counterparts in plants or animals. Thus, the targeting of SD for fungicide design anticipates little off-target toxicity; low levels of off-target toxicity has been reported for carpropamid.[8a]

Our primary goal in this study was the discovery of novel SD inhibitors as leads, which would provide opportunities to supersede the activity of the known inhibitors and their biological efficacies. On the basis of the proposed E1cb-like catalytic mechanism of the enzyme[6b] and the noted inhibitor mimics of the two sp2 centers thought to exist in a transient enol intermediate in the reaction path,[7] we chose to retain the amide functionality found in many potent inhibitors of SD. The amide functionality provides hydrogen bonding interactions with two active-site water molecules, which in turn share hydrogen bonds with the side chains of tyrosines 30 and 50 and histidines 85 and 110.[7,11] More specifically, we sought to discover new carboxylic acids which, when coupled to certain amines known to be well accommodated in the binding pocket of SD,[12] would generate potent enzyme inhibitors and leads for blasticide development. Facilitating the value of this work is a sensitive enzyme assay which employs an alternate substrate that can report true inhibition constants at the picomolar level.[14] In this communication, we report the successful application of combinatorial chemistry and in vitro screening for the identification of a novel inhibitor of SD that has fungicidal properties.



Scheme 1. Preparation of the library of amides targeted at inhibition of scytalone dehydratase

Chemistry

Carboxylic acids for use in the synthesis of SD inhibitors were selected from DuPont's compound collection. Our search identified 21,672 cyclic, aliphatic carboxylic acids. Applying knowledge of the size and electronic nature of the active-site binding pocket of SD,[7,11,12] this list was culled to remove incompatible functionalities and molecules judged to be too large to afford a "short" list of 720 carboxylic acids. These acids were ordered from the corporate compound storage, and the available ones were analyzed by MS (loop injection, APCI- mode) to verify their identity. Ultimately, 192 carboxylic acids were individually coupled

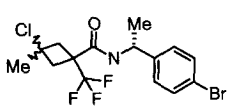
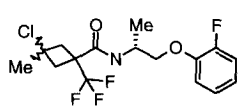
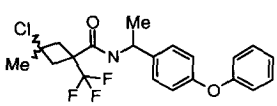
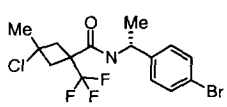
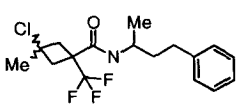
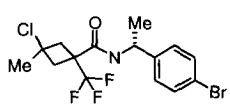
derivative of carbonyldiimidazole, CBMIT (Scheme 1).[15] Reactions (16 h using an orbital shaker for agitation) were run at 25 °C on a 0.4 mmol scale. Work up was performed by using a two-phase extraction between water and dichloromethane. Organic extracts were dried over $MgSO_4$ and concentrated on a speedvac centrifuge under high vacuum. All samples were analyzed by LC-MS to verify that the desired subjects were present.

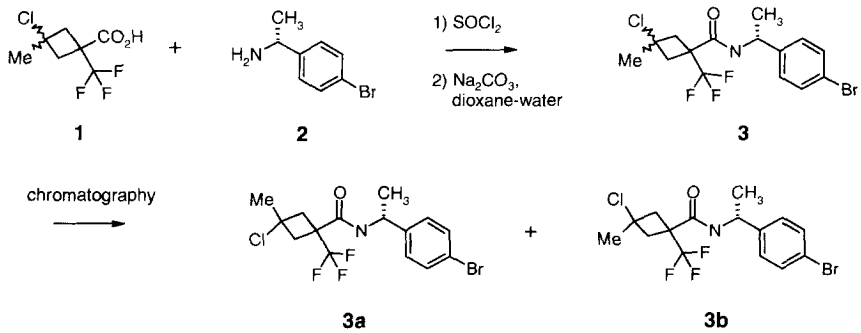
Results and Discussion

Product mixtures (172) were tested in SD inhibition assays.[12a,14] We were most intrigued by the structure of the carboxylic acid used in the synthesis of the second most active pool,[16] and we resynthesized its individual components. The "discretes" were assayed for potency of enzyme inhibition and it was found that **3** has an K_i of 0.049 nM, which approximates that of carpropamid (Table 1). The other amine side chains of the acid of **3** are less potent inhibitors by factors of 16-170. Inhibitor **3** was prepared from carboxylic acid **1**[17] as a mixture of two isomers, which could be separated chromatographically (Scheme 2). The isomer with the chlorine *trans* to the trifluoromethyl group (**3b**) is a 90-fold more potent inhibitor than the *cis* isomer (**3a**). **3b** protected rice plants from infestation by blast upon foliar application with an ED_{90} of 40 ppm.

Co-crystallization of SD with **3b** at neutral pH, data collection, and refinement were achieved by the methods of Wawrzak et al.[7] The crystal structure of **3b** bound to SD was solved at 1.8 Å resolution and it provided an excellent model according to the refinement statistics (R_{factor} = 19.8%, R_{free} = 23.7%) and by visual inspection of the electron density surrounding the inhibitor and the two water molecules (Figures 2 and 3). The crystal structure shows that **3b** binds with the chlorine atom and methyl group oriented towards the ND2 and

Table 1. Inhibition of Scytalone Dehydratase by Cyclobutane Carboxamides

Compd No.	Structure	K_i (nM)	Compd No.	Structure	K_i (nM)
3		0.049	**6**		0.78
4		5.6	**3a**		2.3
5		8.6	**3b**		0.026



Scheme 2. Preparation of isomeric cyclobutane carboxamide SD inhibitors

OD1 atoms of Asn131's side chain providing a complementary electrostatic interaction. There are two crystallographic water molecules in the active site as has been found in other X-ray structures of the enzyme.[7,11] Similar to other structures of SD with inhibitors, the amide N1 of **3b** forms a hydrogen bond to one water molecule which, in turn, is hydrogen bonded to the side chain NE's of His85 and His110. A second hydrogen bonding network is shared between the other active-site water molecule, the Tyr30 and Tyr50 OH's, and the carbonyl of **3b**. However, unlike the interactions of other inhibitors with SD which have been characterized by X-ray crystallography,[7,11] the carbonyl oxygen of **3b** does not hydrogen bond to the water molecule; instead it accepts a hydrogen exclusively from the Tyr50 OH. The side chain of Tyr50 and the water molecule located between the two tyrosines have been analyzed as having more movement than other SD residues.[7] The trifluoromethyl group, phenyl group and bromine atom occupy lipophilic pockets. Distances of key interactions are given in Figure 4. The *R* configuration of the chiral center is critical for good binding and disease control (data not shown).

In conclusion, we have discovered **3b** as a potent inhibitor of SD having a novel structure and which we judge to be an attractive starting point for optimization. **3b** exhibits preventative activity in protecting rice leaves from blast infections in foliar applications, consistent with its mode of action stemming from in situ inhibition of SD. Structure-based selection of amines and deselection of acids was employed to improve the likelihood of discovering new potent inhibitors. A combinatorial approach to synthesizing and testing saved 75% of the effort in comparison to synthesizing and testing each molecule discretely. The binding interactions of **3b** with amino acid side chains and the two water molecules of SD provide new insights for molecular recognition that will be analyzed in more detail elsewhere. We will disclose a full report of the optimization of lead **3b** shortly.

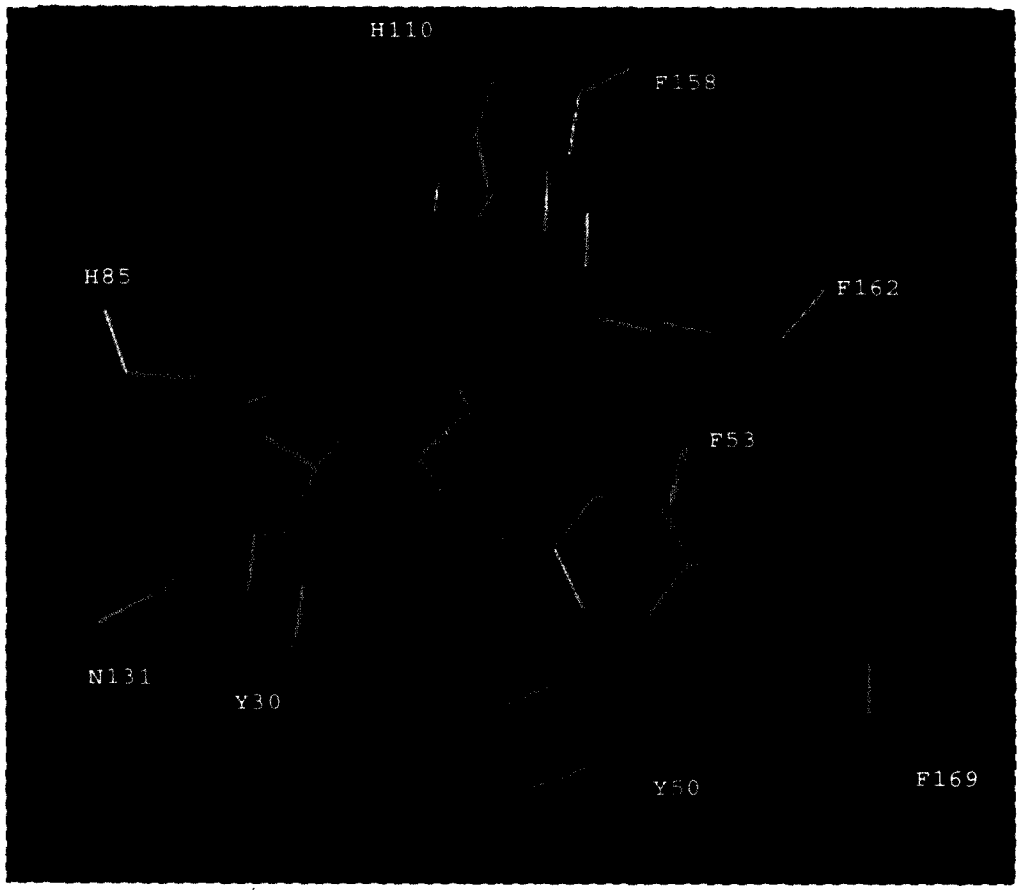


Figure 2. Model of **3b** in the active site of scytalone dehydratase. The red spheres represent water molecules and the yellow dashed lines represent hydrogen bonds.

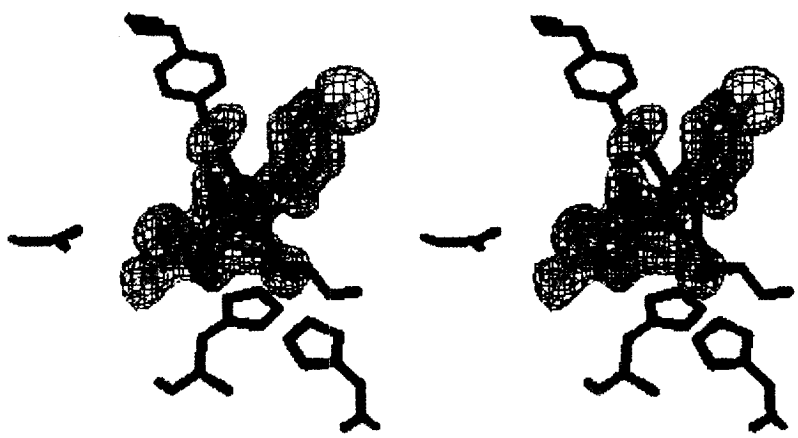


Figure 3. Stereo view of the 2Fo –Fc electron density map of **3b** within the active site of scytalone dehydratase.

Interaction			Distance (Å)
3b-O1	→	Y50-OH	2.6
Y50-OH	→	H_2O	2.8
Y30-OH	→	H_2O	2.8
3b-O1	→	H_2O	4.4
3b-CL1	→	N131-ND2	3.6
3b-C13	→	N131-OD1	3.7
3b N1	→	H_2O	2.9
H85-NE2	→	H_2O	2.9
H110-NE2	→	H_2O	2.9

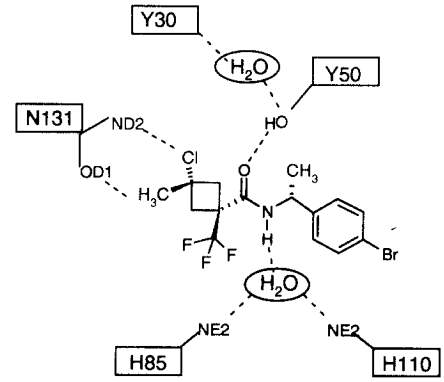
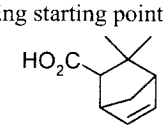


Figure 4. Inter-atomic distances of key interactions found in enzyme-inhibitor complex of SD and **3b**.

References and Notes

1. Present address: Northwestern University, DND-CAT, 9700 S. Cass Ave., Argonne, IL 60439.
2. Present address: DuPont Pharmaceuticals, Bldg. 400, Experimental Station, Wilmington DE 19880-0400.
3. Howard, R. J.; Ferrari, M. A. *Exp. Mycol.* **1989**, *13*, 403.
4. Bell, A. A.; Wheeler, M. H. *Annu. Rev. Phytopath.* **1986**, *24*, 411.
5. Thompson, J. E.; Basarab, G. S.; Andersson, A., Lindqvist, Y.; Jordan, D. B. *Biochemistry* **1997**, *36*, 1852.
6. (a) Jordan, D. B.; Basarab, G. S.; Steffens, J. J.; Lundqvist, T.; Pfrogner, B. R.; Schwartz, R. S.; Wawrzak, Z. *Pesticide Sci.* **1999**, *55*, 277. (b) Basarab, G. S.; Steffens, J. J.; Wawrzak, Z.; Schwartz, R. S.; Lundqvist, T.; Jordan, D. B. *Biochemistry* **1999**, *38*, 6012.
7. Wawrzak, Z.; Sandolova, T.; Steffens, J. J.; Basarab, G. S.; Lundqvist, T.; Lindqvist, Y.; Jordan, D. B. *Proteins: Struct., Funct., Genet.* **1999**, *35*, 425.
8. (a) Kurahashi, Y.; Sakawa, S.; Kinbara, T.; Tanaka, K.; Kagabu, S. *Nippon Noyaku Gakkaishi* **1997**, *22*, 108. (b) Tsuji, G.; Takeda, T.; Furusawa, I.; Horino, O.; Kubo, Y. *Pestic. Biochem. Physiol.* **1997**, *57*, 211.
9. *Agrow*, PJP Publications Ltd.: UK, 1997; Vol. 287, pp 21-22.
10. Sieverding, E.; Hirooka, T.; Nishiguchi, T.; Yamamoto, Y.; Spadafora, V. J.; Hasui, H. In *Proc., The 1998 Brighton Conference — Pests and Diseases*; British Crop Protection Council: Brighton, England, 1998; Vol. 2, pp 359-366.
11. Lundqvist, T.; Rice, J., Hodge, C. N.; Basarab, G. S.; Pierce, J.; Lindqvist, Y. *Structure (London)* **1994**, *2*, 937.
12. (a) Chen, J. M.; Xu, S. L.; Wawrzak, Z.; Basarab, G. S.; Jordan, D. B. *Biochemistry* **1998**, *37*, 17735. (b) Jordan, D. B.; Lessen, T.; Wawrzak, Z., Bisaha, J. J.; Gehret, T. C.; Hansen, S. L.; Schwartz, R. S.; Basarab, G. S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1607. (c) Basarab, G. S.; Jordan, D. B.; Gehret, T. C.; Schwartz, R. S.; Wawrzak, Z. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1613.
13. Andersson, A.; Jordan, D.; Schneider, G.; Lindqvist, Y. *Structure (London)* **1996**, *4*, 1161.
14. Thompson, J. E.; Basarab, G. S.; Pierce, J.; Hodge, C. N.; and Jordan, D. B. *Anal. Biochem.* **1998**, *256*, 1.
15. Saha, A. K.; Schultz, P.; Rapoport, H. *J. Am. Chem. Soc.* **1989**, *111*, 4856.
16. The carboxylic acid component of the most active pool was 3,3-dimethyl-5-norbornene-2-carboxylic acid, **4**, which we judged to not be a promising starting point for optimization. This pool was not deconvoluted.



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17. Hall, H. K., Jr.; Blanchard, E. P., Jr.; Martin, E. L. *Macromolecules* **1971**, *4*, 142.