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DESIGN OF SCYTALONE DEHYDRATASE INHIBITORS AS RICE BLAST FUNGICIDES: (N-PHENOXYPROPYL)-CARBOXAMIDES

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Abstract: Insights gained from a crystal structure of scytalone dehydratase led to the design of carboxamide inhibitors with a phenoxypropyl group substituted on the nitrogen atom. Potent enzyme inhibitors were synthesized around this motif, the best of which provided excellent control of rice blast disease in greenhouse assays and outdoor field trials.

Scytalone dehydratase (SD) is a requisite enzyme in the fungal melanin biosynthetic pathway of the rice blast pathogen, *Magnaporthe grisea*. Fungal melanin, a black biopolymer of 1,8-dihydroxynaphthalene, is localized between the cell wall and cell membrane of a specialized infection organelle that potentiates penetration of the rice leaf.[3,4] Genetic disruption of SD blocks melanin production and pathogenicity by *M. grisea*,[5] and it follows that chemical inhibition of the enzyme target would lead to disease control. Two inhibitors of SD are currently being commercialized for the control of rice blast: carpropamid **1**[6] and diclocymet **2**[7] (Figure 1). The gene encoding SD has been cloned and expressed in *E. coli*,[8] and the X-ray structure of the enzyme bound with salicylamide inhibitor **3** was reported in 1994.[9] The carboxamides of these inhibitors are thought to mimic the enol intermediate proposed in an E1cb-like mechanism for SD.[10,11] More recently, several other X-ray structures of SD complexed with other inhibitors have been solved.[11-13] This structural information can be used to design new classes of inhibitors and rice blast fungicides.

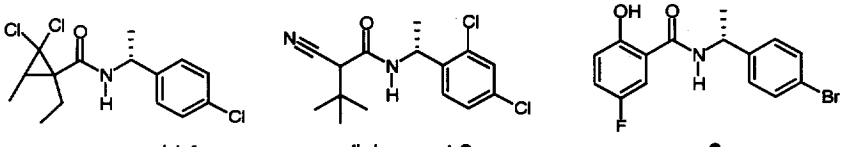

Figure 1. Literature inhibitors of scytalone dehydratase.

Inhibitor design

A common structural feature of SD inhibitors **1**, **2**, and **3** is the N-phenethylcarboxamide group with the *R* absolute configuration. From the original crystal structure of **3** complexed with SD,[9] we set out to design inhibitors with a more extended chain linking the phenyl group to the carboxamide nitrogen. The inhibitor

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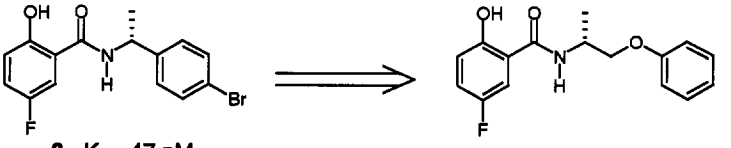
binding site is buried within enzyme requiring an opening and closing of flexible protein residues to allow entry of the inhibitor. We recognized that the region of the binding pocket surrounding the bromine atom of **3** was quite large and could accommodate a phenyl group. Through modeling, we removed the bromine of **3**, slid the phenyl group to which it was attached into the vacated space, and joined the phenyl group to the carboxamide nitrogen with three carbon atoms maintaining the methyl group α to the nitrogen. We then replaced the benzylic carbon atom in the model with an oxygen atom, which lies about 4.3 Å away from the Tyr50 phenol oxygen of SD. Though too far for a hydrogen bond to Tyr50, the ether of the designed phenoxypropyl salicylamide **4** (Scheme 1) was envisioned to lie in a favorable electrostatic environment and to adjust the physical properties of the inhibitor for improved expression of in vivo activity (see below). To minimize the strain energies associated with the modification of **3** to **4**, the model was optimized using a molecular mechanics force field.[14] The new inhibitor is accommodated without significant movement of the active site residues relative to the parallel optimization with **3**. This is graphically represented in Figure 2, where we show a solvent accessible (Connolly) surface of the SD binding pocket surrounding the overlays of **3** and **4** after optimization.

Scheme 1.



3 $K_i = 47$ pM **4** $K_i = 440$ pM

Though **3** is a potent inhibitor of SD, its activity in greenhouse disease control assays is not commensurate. Salicylamides such as **3** were found to be detoxified through conjugation of the phenol with glucose in cultures of *M. grisea* (G. Basarab, unpublished results). Hence, we evolved our strategy to prepare substituted phenoxypropyl carboxamide analogues of **1** and **2** as an extension of our design of salicylamide **4**.

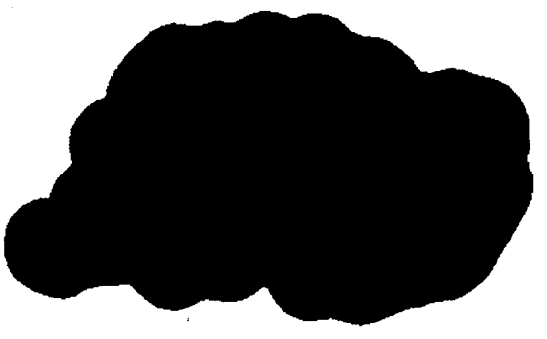
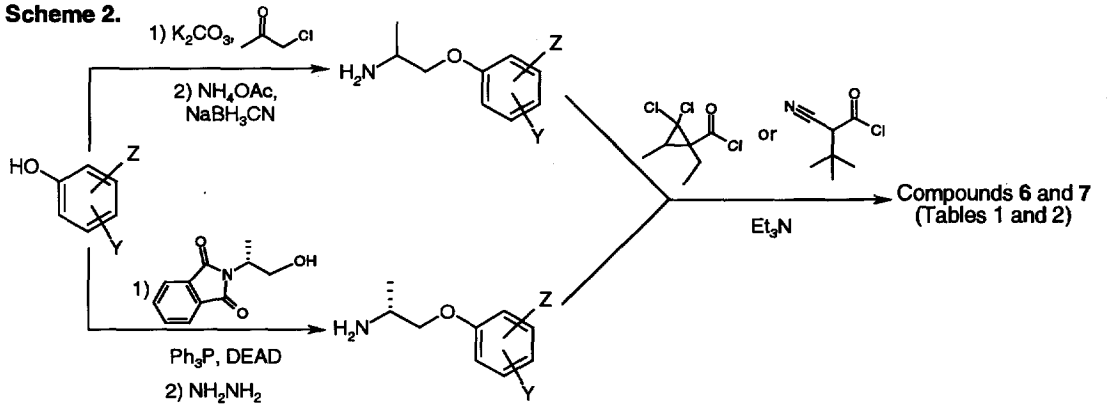


Figure 2. Compounds **3** (red) and **4** (blue) within the "back-half" of the Connolly surface generated for the SD binding pocket.

Chemistry

Racemic phenoxypropyl amines were prepared by treatment of phenols with base and chloroacetone followed by reductive amination. The amines were made chirally by Mitsunobu reaction of *d*- (or *l*-) alaninol phthalimide[15] with phenols followed by treatment with hydrazine (Scheme 2). Acylation with the racemic dichlorocyclopropyl carbonyl chloride of **1**[16] gave the desired carboxamide as a 1:1 mixture of diastereomers that could not be routinely separated. Acylation with the racemic *t*-butyl cyanoacetyl chloride of **2**[17] gave a 1:1 mixture of diastereomers that were readily separated by chromatography.

Scheme 2.

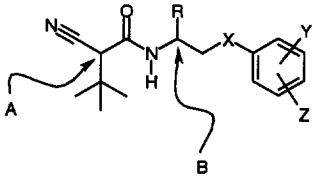


Results and Discussion

Tables 1 and 2 summarize the enzyme and biological data for the cyclopropyl carboxamides and cyanoacetamides, respectively. For the sake of comparison, we included examples of diastereomeric mixtures, single diastereomers and single enantiomers. K_i values were determined as previously described.[12] The adjusted K_i's reflect the number of isomers in the particular preparation as the preponderance of activity resides in one isomer. For example, the single enantiomer **6f** is 4–5 times more active than **6c**, which is an equimolar mixture of all four possible diastereomers, and 2 times more active than **6e**, which is its racemate. ED_{90} is the compound concentration affording 90% disease control as determined from multiple rate foliar rice blast assays.[18]

Structure–activity trends for SD inhibition by the two classes of carboxamides parallel one another. The methyl substituent on the carbon bonded to the carboxamide nitrogen atom increases inhibitory potency largely by decreasing the entropy of the system: first, through the resultant increase in lipophilicity, and second, by decreasing the rotational degrees of freedom in the chain connecting the carboxamide to the phenyl group. The all carbon three-atom bridged compounds **6b** and **7b** are less active than the respective compounds **6c** and **7d** with an oxygen atom in the bridge. The binding pocket of SD is hydrophobic and prefers more lipophilic ligands due to the favorable energetic contributions from desolvation.[19] Therefore, the compounds with the oxygen atom

Table 1. Cyanoacetamide inhibitors of scytalone dehydratase.

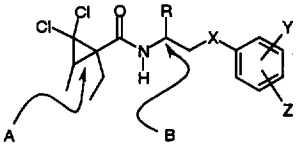


Compound	# of Isomers	Configuration at A	Configuration at B	R	X	Y, Z	SD K_i (nM)	Adjusted K_i (nM)	ED_{90} (mg/L)
6a	2	*R,S*	-	H	CH_2	-	13.6 ± 0.3	6.8	200
6b	4	*R,S*	*R,S*	CH_3	CH_2	-	7.2 ± 0.4	1.8	>200
6c	4	*R,S*	*R,S*	CH_3	O	-	2.8 ± 0.1	0.7	200
6d	2	*rel-S*	*rel-R*	CH_3	O	-	66 ± 1	33	>200
6e	2	*rel-R*	*rel-R*	CH_3	O	-	1.3 ± 0.1	0.66	100
6f	1	*R*	*R*	CH_3	O	-	0.58 ± 0.04	0.58	25
6g	2	*R,S*	*R*	CH_3	O	4-F	1.7 ± 0.1	0.83	100
6h	4	*R,S*	*R,S*	CH_3	O	2-F	0.24 ± 0.01	0.059	50
6i	1	*R*	*R*	CH_3	O	2-F	0.080 ±0.005	0.080	25
6j	2	*rel-R*	*R,S*	CH_3	O	3-F	0.46 ± 0.01	0.23	25
6k	1	*R*	*R*	CH_3	O	3-F	0.24 ± 0.01	0.24	25
6l	1	*R*	*R*	CH_3	O	2,3-di-F	0.95 ± 0.02	0.95	25
6m	1	*S*	*S*	CH_3	O	2,5-di-F	97 ± 10	97	>200
6n	2	*R,S*	*R*	CH_3	O	2,5-di-F	0.042 ± 0.006	0.021	6.2
6o	1	*S*	*R*	CH_3	O	2,5-di-F	0.73 ± 0.01	0.73	6.2
6p	1	*R*	*R*	CH_3	O	2,5-di-F	0.020 ± 0.004	0.020	0.8
6q	1	*R*	*S*	CH_3	O	2,5-di-F	7.7 ± 0.9	7.7	>200

overcome the inherent desolvation disadvantage in accord with the electrostatic environment provided by the Tyr50 hydroxyl in the binding pocket. Conservative substitution of a fluorine atom *ortho* or *meta* on the phenoxy group (**6h**, **6j**, and **7h**) increases SD inhibitory potency while substitution *para* (**6g**, **7f**) decreases potency. From our model, the *para* position borders the wall of the SD binding cavity bounded by the C-terminal arm of the enzyme. Larger halogen (and other lipophilic) substituents at the *ortho* and *meta* positions afford SD inhibitor activities approximating the fluorine substituent (data not shown), but activities in greenhouse assays are compromised. Polar substituents (not shown) reduce activity against SD, which is understandable due to the hydrophobic environment of the binding cavity and the smaller contribution to binding derived from the energies of desolvation. Increased potency against SD with fluorine at the *ortho* and *meta* positions is additive as seen with the 2,5-difluoro substitution pattern (compare **6p** to **6i** and **6k** and **7j** with **7g** and **7h**). The alternative 2,3-difluoro substitution pattern (**6l** and **7i**) diminished SD inhibitory activity.

Inhibition of SD correlates with the phenoxypropyl group *R* configuration as **6p** and **7j** were about 400-fold more potent than **6q** and **7k**, respectively. The K_i's for the less active isomers **6q** and **7k** reflect, for the most part, the 97% ee of the precursor *l*-alaninol used in their preparations. Inhibitors **6** with the *R* configuration in the cyanoacetic acid fragment bind SD tighter than those with the *S* configuration (compare **6e** with **6d** and **6p**

Table 2. Cyclopropane carboxamide inhibitors of scytalone dehydratase.



Compound	# of Isomers	Configuration at A	Configuration at B	R	X	Y, Z	SD K_i (nM)	Adjusted K_i (nM)	ED_{90} (mg/L)
7a	2	*rel-R,R*	-	H	CH_2	-	1.9 ± 0.3	0.96	30
7b	4	*rel-R,R*	*R,S*	CH_3	CH_2	-	0.83 ± 0.01	0.21	30
7c	2	*rel-R,R*	-	H	O	-	7.5 ± 0.4	3.7	200
7d	4	*rel-R,R*	*R,S*	CH_3	O	-	0.38 ± 0.02	0.094	50
7e	2	*rel-R,R*	*R*	CH_3	O	-	0.17 ± 0.01	0.0835	30
7f	2	*rel-R,R*	*R*	CH_3	O	4-F	0.22 ± 0.01	0.11	30
7g	2	*rel-R,R*	*R*	CH_3	O	2-F	0.37 ± 0.05	0.19	50
7h	2	*rel-R,R*	*R*	CH_3	O	3-F	0.11 ± 0.01	0.057	30
7i	2	*rel-R,R*	*R*	CH_3	O	2,3-di-F	2.2 ± 0.1	2.2	100
7j	2	*rel-R,R*	*R*	CH_3	O	2,5-di-F	0.085 ± 0.009	0.042	6
7k	2	*rel-R,R*	*S*	CH_3	O	2,5-di-F	33 ± 1	16	100

with **6o**). The less active diastereomer may well be contaminated by trace amounts of the more active one, the latter being undetected by the limits of NMR analysis. Diastereomer **6o** shows good activity in the greenhouse rice blast assay, which would not be expected based on its K_i and which is very likely due to epimerization to the more active diastereomer **6p** during the course of the week-long in vivo assay. Assignment of the *R* configuration for the more active diastereomer **6p** was determined by X-ray diffraction (see below). The configuration responsible for inhibitory activity of the cyclopropane carboxylic acid of compounds **7** has previously been assigned as *R,R*.[11,12] Since there is no capability for epimerization, the less active *S,S* cyclopropane diastereomer does not show significant in vivo activity.

An X-ray structure of SD in complex with **6p** was solved to 1.9 Å resolution via described methods[11,13] for crystallization, data collection and refinement.[20] Electron density for the inhibitor in the binding pocket was excellent. The *R,R* absolute configuration of the inhibitor was readily apparent and the extended conformation of the phenoxypropyl carboxamide confirmed the prediction. The hydroxyl of Tyr50 lies 3.7 Å from the ether oxygen of **6p**. The nitrile nitrogen lies 3.2 Å from the side chain carboxamide of Asn131 in line for an albeit weak hydrogen bond. There are two active site water molecules in hydrogen bonding distance to the oxygen and nitrogen of the carboxamide of **6p**, which are similarly present in previous X-ray structure determinations of SD-inhibitor complexes.[9,11,12] The two water molecules are, in turn, hydrogen bonded to four residues of the enzyme: Tyr30, Try50, His85, and His110. Otherwise, the binding of **6p** with SD is driven by the entropic contributions that result from moving a relatively hydrophobic molecule from the aqueous environment to a lipophilic enzyme binding site. The enzyme surrounds **6p** with the majority of contacts coming from the side chains of hydrophobic residues. The disruption of the ordered solvent sphere around less water soluble (more lipophilic) chemistry as

represented by **6p** favors the association with the enzyme over more water soluble chemistry. However, excessive lipophilicity generally decreases bio-availability and limits movement through the plant vascular system,[21] and there should therefore be a measure of caution when a designed increase in binding potency is due to increased lipophilicity alone.

In summary, potent inhibitors of SD were designed and synthesized. The design concept was verified by X-ray crystallography. It was important to optimize not just for enzyme potency but also for physical properties in order to maximize fungicidal activity in greenhouse assays, and the original X-ray structure[9] inspired ideas for both aspects. Due to superior activities in greenhouse assays, **6p** and **6n** (the latter being the 1:1 mixture of **6o** and **6p** since epimerization occurs in vivo) were examined for rice blast control in field trials. Disease control furnished by **6p** and **6n** was equal to or better than those of **1** and **2** in side-by-side comparisons.

References and Notes

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