Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 491–494

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Selection of a Potent Inhibitor of Trihydroxynaphthalene Reductase by Sorting Disease Control Data

Der-Ing Liao,[a] Gregory S. Basarab,[a] Anthony A. Gatenby[a] and Douglas B. Jordan[b,*]

[a]*DuPont Central Research and Development, Experimental Station, Wilmington DE, 19880, USA*
[b]*DuPont Pharmaceutical Company, Stine Haskell Research Center, PO Box 30, Newark DE 19714, USA*

Received 16 November 1999; accepted 12 January 2000

Abstract—Compounds that control rice blast, but not other crop diseases, were selected for testing as inhibitors of trihydroxynaphthalene reductase of the fungal melanin biosynthetic pathway. A potent inhibitor of the enzyme (**2**) ($K_i = 25$ nM) was identified. An X-ray structure of the enzyme-NADPH-**2** complex was determined at 2.1 Å resolution.

Enzymes of fungal melanin biosynthesis are fertile ground for the discovery of inhibitors useful for the control of blast disease in rice. Trihydroxynaphthalene reductase (3HNR) is the biochemical target of tricyclazole[1] ($K_i = 24$ nM),[2] pyroquilon[3] ($K_i = 14$ nM),[4] and phthalide[5] ($K_i = 2.0$ nM),[4] effective agents available for preventing the crop disease (Fig. 1). Carpropamid,[6,7] a potent inhibitor ($K_i = 30$ pM)[8] of scytalone dehydratase (SD), has been recently commercialized and two additional inhibitors of SD are currently under development.[9,10] There is one X-ray structure of 3HNR in complex with tricyclazole and NADPH[11] and several of the SD-inhibitor complexes.[8,12–17] Examples of structure-based design of potent SD inhibitors with blasticide activity have been reported.[13–16] 3HNR and SD are particularly attractive targets for fungicide design because the fungal melanin biosynthetic pathway does not exist in off-target organisms; the commercial blasticides shown in Figure 1 are notable for their safety to off-target organisms.[6,18]

Fungal melanin biosynthesis includes a pentaketide pathway that links acetate units to produce 1,3,6,8-tetrahydroxynaphthalene.[19] Through a series of two reductions and two dehydrations, tetrahydroxynaphthalene is transformed to 1,8-dihydroxynaphthalene, presumably the ultimate precursor of the polymeric fungal melanin. Fungal melanin is employed in the initiation of disease whereby the pathogen develops and focuses enough turgor pressure to punch itself through the leaf cells of the host plant.[20,21] Among plant diseases, rice blast stands out as the economically most important one requiring melanin biosynthesis for infection. From over two decades of whole-plant screening for fungicides at DuPont, rice blast is the only melanin-dependent disease that has been incorporated into routine assays. In view of this singularity, we sought to find melanin biosynthesis inhibitors by searching our database for compounds that control rice blast but lack significant activity against other plant diseases, the purpose of this selection strategy being to identify potential inhibitors of 3HNR and SD.

Results and Discussion

Selection and screening

Biological data for nearly 400,000 compounds in the DuPont data warehouse were searched for those having >90% control of rice blast and <80% control of the other fungal diseases reported (wheat powdery mildew, grape downy mildew, grape powdery mildew, tomato late blight, wheat leaf rust, wheat glume blotch, wheat foot rot, apple scab, potato late blight, and rice sheath blight). The search generated a list of 1000 compounds putatively enriched in those that inhibit fungal melanin biosynthesis. Over 150 compounds were eliminated from the list because they, indeed, had been prepared as inhibitors of SD and 3HNR in directed fungicide design programs. The remaining list was further culled to eliminate samples with unknown structures (e.g. natural product extracts). Though a large number of the

*Corresponding author. Tel.: +1-302-451-0075; fax: +1-302-366-5738; e-mail: doug.b.jordan@usa.dupont.com

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00037-8

remaining candidates were depleted, 390 were still in storage; these were obtained and screened against the catalytic activities of 3HNR[2] and SD[13,22] using published procedures.[23] The results of the survey indicate that two potent inhibitors were among the collection of compounds: **1**, which has a K_i of 6.4 nM against SD, and **2**, which has a K_i of 25 nM against 3HNR (Fig. 2). A number of other inhibitors considerably less potent than **1** and **2** were identified, and these were not further pursued. The inhibition constant for **2** is in the range of the most potent inhibitors known for 3HNR, and it is therefore of greater interest than compound **1**, whose K_i value is 1000-fold less potent than the best SD inhibitors. Nanomolar K_i values for inhibitors are known to translate into in vivo activity (control of rice blast) for the reductase, while picomolar K_i values are required to do so for the dehydratase. It is possible that there are inhibitors of fungal melanin biosynthetic enzymes other than the enzymes screened (3HNR and SD) among the rice blast selective compounds surveyed; e.g. there may exist, within the surveyed compounds, inhibitors of the enzyme(s) responsible for condensing acetate units to yield 1,3,6,8-tetrahydroxynaphthalene.[19]

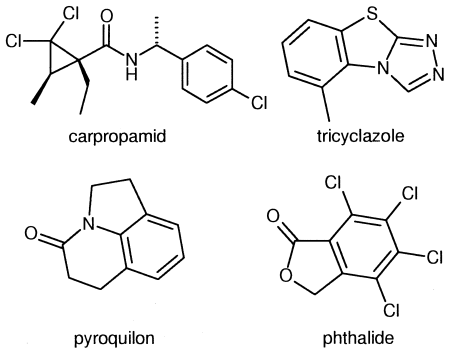

Figure 1. Commercial blasticides that target melanin biosynthesis. Capropamid inhibits SD, and the others inhibit 3HNR.

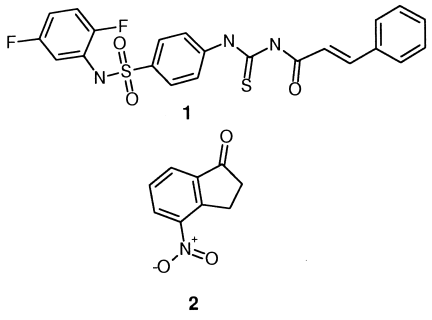

Figure 2. Inhibitors of SD (**1**) or 3HNR (**2**) selected through the screening strategy.

The original crystal structure of 3HNR in complex with tricyclazole and NADPH (PDB accession code 1YBV)

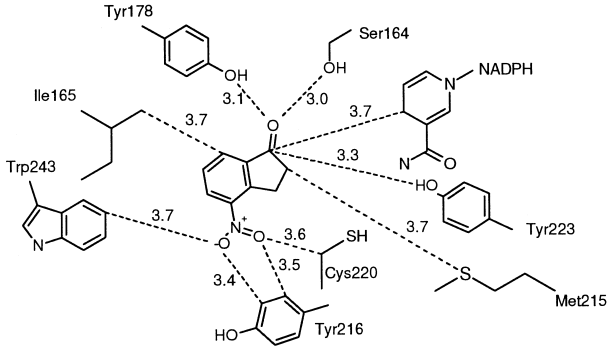

Figure 4. Interactions of **2** within the active site of 3HNR. Distances are in Å.

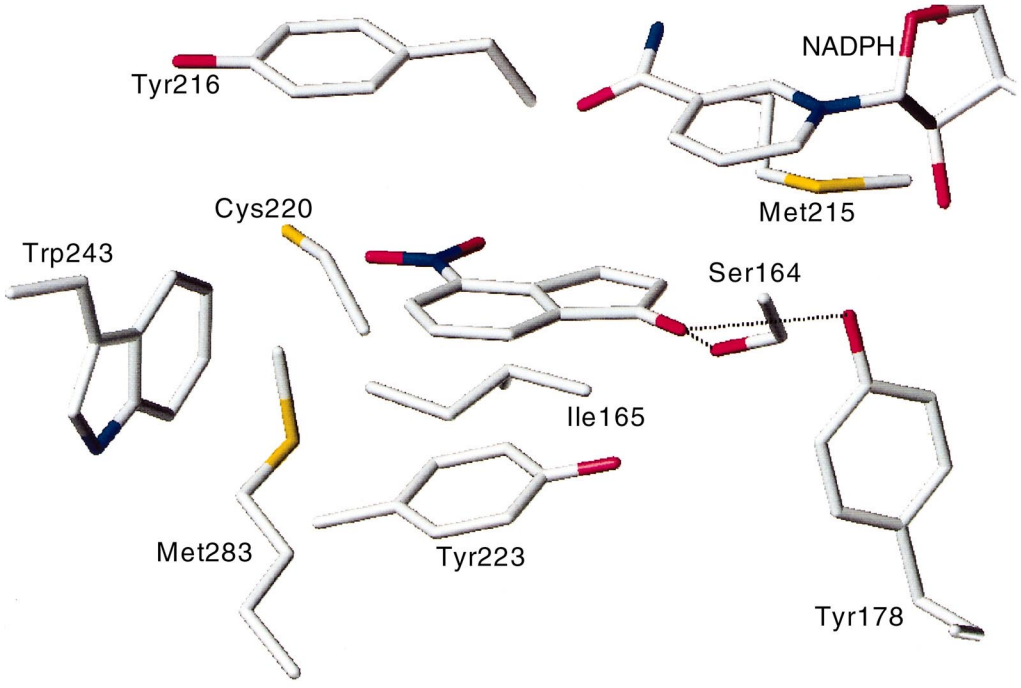

Figure 3. Binding of **2** within the active site of 3HNR. Hydrogen Bonds are indicated.

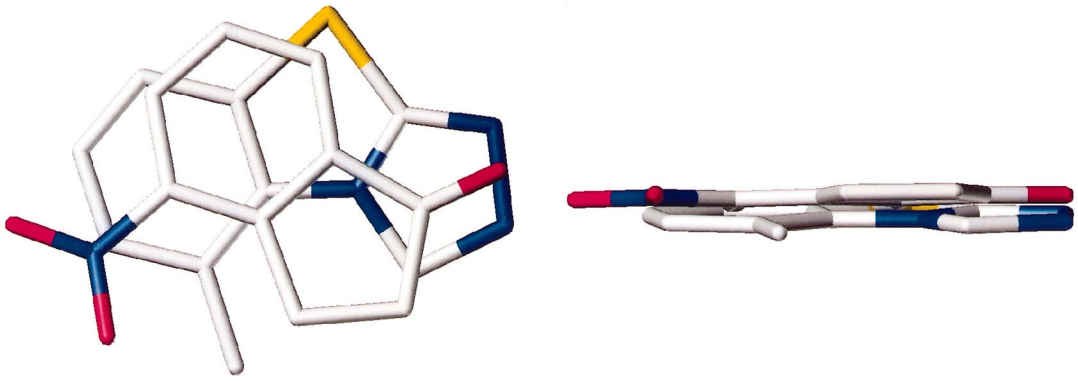

Figure 5. Overlays of **2** and tricyclazole from their orientations within the active site of 3HNR.

clearly indicates two hydrogen bonds from the hydroxyl groups of Ser164 and Tyr178 to the two adjacent imidazole nitrogen atoms of the inhibitor.[11] It was unclear whether the nitro group or the carbonyl oxygen of **2** would be oriented for sharing hydrogen bonds with the hydroxyl groups of Ser164 and Tyr178. For that matter, it was considered that **2** may occupy an altogether different binding site than tricyclazole. Because of the uncertainties regarding the orientation of **2** within 3HNR, a study to solve the three-dimensional structure was initiated.

The X-ray structure of 3HNR-NADPH-2

Co-crystallization of 3HNR with **2** and NADPH, data collection, and refinement were conducted by the methods of Andersson et al.[11] with some modifications.[24] The structure (PDB accession code 1DOH), solved at 2.1 Å resolution, provides an excellent model (Fig. 3) according to the refinement statistics and by visual inspection of the electron density surrounding the inhibitor, NADPH and the protein. The resolution improves upon that of the original 3HNR structure reported at 2.8 Å. The crystal structure shows that **2** is sandwiched between the nicotinamide ring of NADPH and the phenol ring of Tyr223. Its carbonyl oxygen is oriented towards the hydroxyl groups of Ser164 and Tyr178 for accepting hydrogen atoms. The nitro group is pointed towards a lipophylic region of the active site occupied by Tyr216 and Cys220. Distances of key interactions are listed in Figure 4. When the new structure is superimposed on that of 3HNR complexed with tricyclazole and NADPH,[11] the two inhibitors occupy nearly the same space (Fig. 5). Most notably the two inhibitors, which are flat molecules, lie nearly co-planar to one another in three-dimensional space; the carbonyl oxygen of **2** bisects the bond connecting the adjacent nitrogen atoms of tricyclazole. There is little movement of amino acid side residues involved in inhibitor interactions between the two crystal structures.

Conclusions

A strategy for enriching a collection of compounds with inhibitors of melanin biosynthetic enzymes was devised and executed. The selection procedure was proven effective in that it retrieved inhibitors of 3HNR and SD that had been synthesized in design programs. In general, the strategy may be considered as a short cut for finding biologically active molecules for specific biochemical targets. The fact that only two novel and potent inhibitors of the two enzymes were found indicates that the collection of compounds screened is not a rich source of such molecules. Nonetheless, inhibitor **2** is of high interest for follow-up synthesis because its K_i compares favorably with those of commercial blasticides. The 3HNR–NADPH-**2** complex is the second inhibitor structure reported for the enzyme. The new structure adds a measure of confidence for making predictions for the binding of other inhibitors to 3HNR, including those derived from the design of future generations of inhibitors.

References and Notes

1. Tokousbalides, M. C.; Sisler, H. D. *Pestic. Biochem. Physiol.* **1978**, *8*, 26.
2. Thompson, J. E.; Basarab, G. S.; Andersson, A.; Lindqvist, Y.; Jordan, D. B. *Biochemistry* **1997**, *36*, 1852.
3. Woloshuk, C. P.; Wolkow, P. M.; Sisler, H. D. *Pestic. Sci.* **1981**, *12*, 86.
4. Values were determined for this work.
5. Ishida, M.; Nambu, K. *Noyaku Kagaku* **1975**, *3*, 10.
6. Kurahashi, Y.; Sakawa, S.; Kinbara, T.; Tanaka, K.; Kagabu, S. *Nippon Noyaku Gakkaishi* **1997**, *22*, 108.
7. Tsuji, G.; Takeda, T.; Furusawa, I.; Horino, O.; Kubo, Y. *Pestic. Biochem. Physiol.* **1997**, *57*, 211.
8. Wawrzak, Z.; Sandolova, T.; Steffens, J. J.; Basarab, G. S.; Lundqvist, T.; Lindqvist, Y.; Jordan, D. B. *Proteins: Struct., Funct., Genet.* **1999**, *35*, 425.
9. In *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. Andersson, A.; Jordan, D.; Schneider, G.; Lindqvist, Y. *Structure (London)* **1996**, *4*, 1161.
12. Lundqvist, T.; Rice, J.; Hodge, C. N.; Basarab, G. S.; Pierce, J.; Lindqvist, Y. *Structure (London)* **1994**, *2*, 937.
13. Chen, J. M.; Xu, S. L.; Wawrzak, Z.; Basarab, G. S.; Jordan, D. B. *Biochemistry* **1998**, *37*, 17735.
14. Basarab, G. S.; Steffens, J. J.; Wawrzak, Z.; Schwartz, R. S.; Lundqvist, T.; Jordan, D. B. *Biochemistry* **1999**, *38*, 6012.

15. Jordan, D. B.; Lessen, T.; Wawrzak, Z.; Bisaha, J. J.; Gehret, T. C.; Hansen, S. L.; Schwartz, R. S.; Basarab, G. S. *Biorg. Med. Chem. Lett*. **1999**, *9*, 1607–1612.
16. Basarab, G. S.; Jordan, D. B.; Gehret, T. C.; Schwartz, R. S.; Wawrzak, Z. *Biorg. Med. Chem. Lett*. **1999**, *9*, 1613.
17. Jennings, L. D.; Wawrzak, Z.; Amorose, D.; Schwartz, R. S.; Jordan, D. B. *Biorg. Med. Chem. Lett*. **1999**, *9*, 2509.
18. *The Pesticide Manual*, 11th ed., British Crop Protection Council: Farnham, UK, 1997.
19. Bell, A. A.; Wheeler, M. H. *Annu. Rev. Phytopath*. **1986**, *24*, 411.
20. Howard, R. J.; Ferrari, M. A. *Exp. Mycol*. **1989**, *13*, 403.
21. Bechinger, C.; Giebel, K.-F.; Schnell, M.; Leiderer, P.; Deising, H. B.; Bastmeyer, M. *Science* **1999**, *285*, 1896.
22. Thompson, J. E.; Basarab, G. S.; Pierce, J.; Hodge, C. N.; Jordan, D. B. *Anal. Biochem*. **1998**, *256*, 1.
23. The initial screening of the compounds was at 1 ppm against the catalytic activities of 3HNR and SD of *Magnapothe grisea* (the rice blast pathogen). Carpropamid and tricyclazole were included as positive controls for the SD and 3HNR assays, respectively.
24. The X-ray diffraction data were collected at −168 °C on a Raxis-IV imaging plate system. Molecular replacement and initial refinements were performed with NADPH and the inhibitor removed.