Available online at www.sciencedirect.com

SCIENCE DIRECT

Bioorganic & Medicinal Chemistry Letters 14 (2004) 55–58

Bioorganic & Medicinal Chemistry Letters

Discovery of a novel series of DHODH inhibitors by a docking procedure and QSAR refinement

Johann Leban,* Wael Saeb, Gabriel Garcia, Roland Baumgartner and Bernd Kramer

4SC AG, Am Klopferspitz 19a, 82152 Martinsried, Germany

Received 13 June 2003; revised 18 August 2003; accepted 13 October 2003

Abstract—A novel series of DHODH inhibitors was developed based on a lead which was obtained by a docking procedure and a medicinal chemistry exploration. The activity of the initial lead was improved by a QSAR method to yield low nanomolar inhibitors.

Dihydroorotate dehydrogenase (DHODH) catalyzes the conversion of dihydroorotate to orotate, which might be the rate-limiting step in the pyrimidine biosynthesis.[1] Inhibitors of DHODH show immunosuppressant and antiproliferative activity, which is most pronounced on T-cells.[2] Two examples of inhibitors that have been in clinical development are brequinar and leflunomide. The latter is used in the treatment of methotrexate refractive rheumatoid arthritis.[3] These structural classes of DHODH inhibitors were discovered before DHODH protein was produced in a soluble form, therefore these structures were found by serendipity and their mechanism of action was only found out later.

While several reports deal with novel DHODH inhibitors only two suggest to find novel classes of DHODH inhibitors[4] using the X-ray structure of DHODH.

Our strategy to develop DHODH inhibitors was based on the published crystal structure of the human enzyme.[5] Docking of a library of commercially available compounds using our proprietary in silico screening technology (4Scan®) yielded compounds with a structure that has similarities to brequinar[6] and redoxal,[7] but is a novel chemotype. This class of compounds comprises cyclic aliphatic dicarboxylic acids, in which one carboxylic group is amide bound with an aromatic biphenylaniline. By synthesizing and testing a few analogues, we found that the optimal ring size was a pentacyclic ring with vicinal carboxyl groups separated by a double bond. This refinement yielded the lead compound **1** with an inhibitory activity of 410 nM (IC_{50}) against human DHODH, being comparable to the active metabolite of Leflunomide,[8] A771726 (compound **30**, Table 1).

Docking of **1** into the ubiquinone binding site of the crystal structure of DHODH resulted in a positioning similar to that of brequinar as published: The carboxylic group forms an ion bond to Arg-136 of the enzyme and the biphenyl residue reaches into the hydrophobic pocket.

The compounds were synthesized according to a published method.[9] Substituted biarylanilines were obtained by the Suzuki cross coupling procedure[10] using aromatic boronic acids and halo anilines with Pd and KF in methanol (Fig. 1).[14]

Inhibition and IC_{50} values were determined (was measured) in an in vitro enzyme assay[11] using N-terminally truncated recombinant human DHODH.[12] The assay is based on coupling the ubiquinone reduction to the redox dye 2,6-dichlorphenolindophenol (DCIP) as described. The reduction of DCIP was monitored photometrically by a decreasing absorbtion at 600 nm.

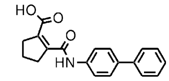
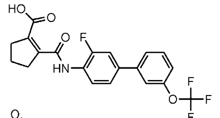
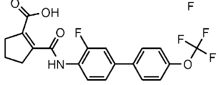
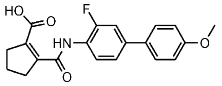
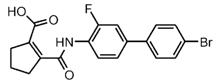
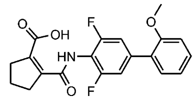
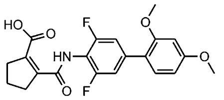
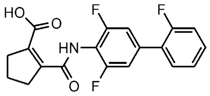
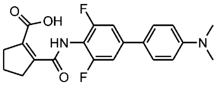
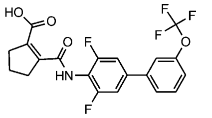
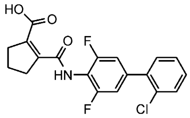
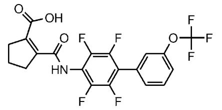
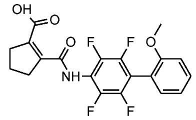
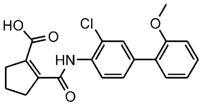
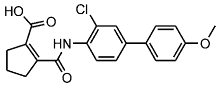
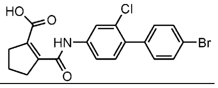
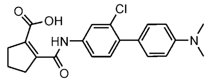
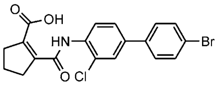
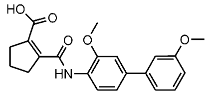
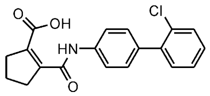
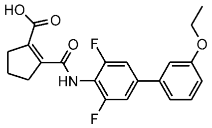
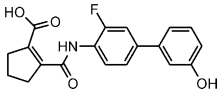
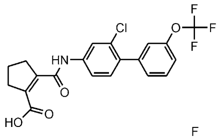
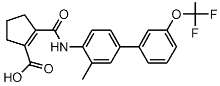
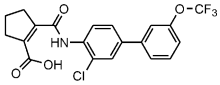
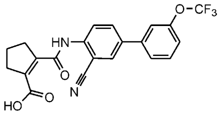
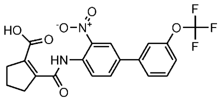
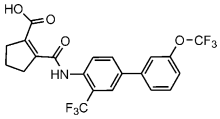
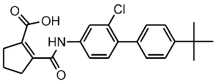
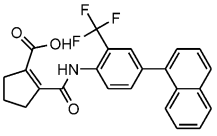
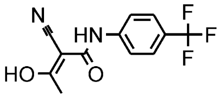
To improve the activity of the lead compound **1**, we envisioned the introduction of small side chains into the biphenyl ring to obtain additional interactions in the hydrophobic pocket of DHODH. To avoid the synthesis of a large number of analogues in a classic medicinal chemistry fashion, we used a computational method to screen a virtual library of several thousand structures

---

* Corresponding author. Fax: +49-89-700-76328; e-mail: leban@4sc.com

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.10.021

Table 1. Inhibition on human DHODH

Compd	DHODH inhibition IC_{50}, nM[a]	Compd structure
1	410	
2	280	
3	5700	
4	1700	
5	735	
6	42	
7	90	
8	45	
9	610	
10	33	
11	18	
12	7	
13	8	
14	310	
15	8400	
16	70	
17	50	
18	2900	
19	170	
20	225	
21	17	
22	610	
23	20	
24	150	
25	290	
26	370	
26	390	
27	840	
28	80	
29	180	
30	435	

[a] Values are means of three experiments, errors are usually around 10%.

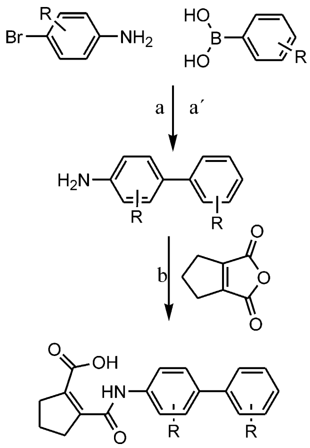


Figure 1. Reagents and conditions: (a) Pd, MeOH, KF, reflux, 8 h; (a′) Pd-tetrakis, DME/water (75/25), Cs_2CO_3, reflux, 8 h; (b) DCM, rt, 16 h.

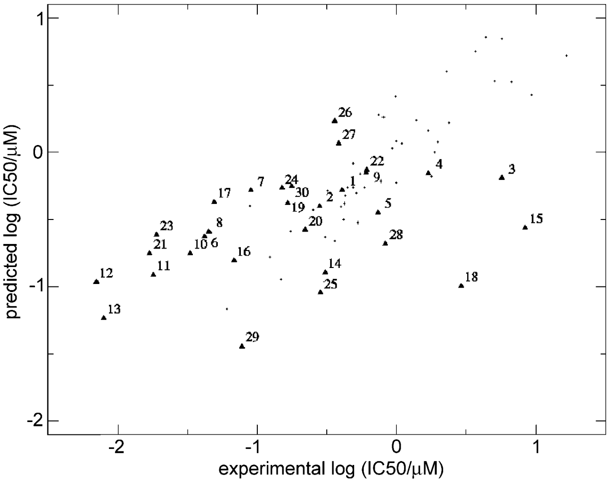


Figure 2. Calculated versus measured IC_{50} of enzyme inhibition. Compounds listed in Table 1 were numbered.

with the core scaffold of **1** and bearing various substituents on the aromatic rings. First, a training set was generated by synthesis and biological testing of 55 compounds was performed. These subset of compounds was sufficiently diverse to allow the setup of a QSAR model. Each molecule was characterized by seventy 2D and internal coordinate dependent 3-D descriptors delivered from the MOE package.[13] Partial least square analysis (PLS) of a subset of 44 molecules and their $\log(IC_{50})$ lead to a cross-validated correlation coefficient of 0.60 and a root mean square error of 0.36. The optimal number of components used was seven. The resulting QSAR model allowed us the prediction of $\log(IC_{50})$ values for the complete virtual library and the selection of 30 high scoring compounds for subsequent synthesis. Their average logarithmic activity was 1–2-fold higher, compared to the training set. Seven new molecules showed activities even beyond (higher than) the training set, and a new lead compound, 80 times more active than **1**, emerged (Fig. 2).

The analysis of the QSAR model gave further hints for important features of a good DHODH inhibitor: Activity increased with rising total van der Waals surface area as well as negatively charged and hydrophobic van der Waals surface area. Negative contributions were attributed to increased molecular weight and volume.

To shed further light on the binding mode of these compounds we co-crystallized the compounds with truncated human DHODH and solved the structure. The compounds have been localized in the binding site according to the expectation, however subtle differences in binding between compounds make some bind more like A771726 and others like brequinar (unpublished observation). We are also in the process to characterize the kinetic of the enzyme inhibition. Analogues with heteroatom substitutions in the pentacyclic ring, side chains on the pentacyclic ring and other hydrophobic residues in place of the biphenylic ring system were also prepared and tested. These findings and details of biological data will be presented in future publications.

In conclusion, we used a structure-based, combined in silico and medicinal chemistry approach to discover novel and potent inhibitors of human DHODH. Such compounds promise to become useful therapeutic agents to treat autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, lupus erythematosus and ulcerative colitis.

Acknowledgements

We thank Martin Kralik, Jan Mies, Tanja Wieber, Heike Endreß and Larissa Bahrjanyj for technical assistance, Matthias Dormeyer, Michael Gassen and Kristina Wolf for helpful discussions, Gerhard Keilhauer and Daniel Vitt for support.

References and notes

1. Jöckel, J.; Wendt, B.; Löffler, M. *Biochem. Pharmacol.* **1998**, *56*, 1053.
2. Fairbanks, L. D.; Bofill, M.; Ruckemann, K.; Simmonds, H. A. *J. Biol. Chem.* **1995**, *270*, 29682.
3. Alldred, A.; Emery, P. *Exp. Opin. Pharmacother.* **2001**, *2*, 125.
4. Sutton, A. E.; Clardy, J. *Tetrahedron Lett.* **2001**, *42*, 547. Pospisil, P.; Kuoni, T.; Scapozza, L.; Folkers, G. J. *Recept. Signal Transduction Res.* **2002**, *22*, 1, 141–154.
5. Liu, S.; Neidhardt, E. A.; Grossman, T. H.; Ocain, T.; Clardy, J. *Struct. Fold Des.* **2000**, *8*, 25.
6. McLean, J. E.; Neidhardt, E. A.; Grossman, T. H.; Hedstrom, L. *Biochemistry* **2001**, *40*, 2194.
7. Knecht, W.; Löffler, M. *FEBS Lett.* **2000**, *467*, 27.
8. Davis, J. P.; Cain, G. A.; Pitts, W. J.; Magolda, R. L.; Copeland, R. A. *Biochemistry* **1996**, *35*, 1270.
9. deJulian-Ortiz, J. V.; Galvez, J.; Munoz-Collado, C.; Garzia-Domenech, R.; Garcia-Domenech, R.; Gimeno-Cardona, C. *J. Med. Chem.* **1999**, *42*, 3308.

10. Suzuki, A. *J. Organometallic Chem.* **1995**, *576*, 147.
11. Neidhardt, E. A.; Punreddy, S. R.; McLean, J. E.; Hedstrom, L.; Grossman, T. H. *J. Mol. Microbiol. Biotechnol.* **1999**, *1*, 183.
12. Ullrich, A.; Knecht, W.; Fries, M.; Löffler, M. *Eur. J. Biochem.* **2001**, *268*, 1861.
13. Brady, G. P., Jr.; Stouten, P. F. *J. Comput. Aided Mol. Des.* **2000**, *14*, 383.
14. All compounds were characterized by MS and NMR (300 MHz) and exhibited satisfactory properties. Examples are given for a few compounds.
    1 $\delta = 1.93$ (m_C, 2H, CH_2), 2.66 (m_C, 2H, CH_2), 2.79 (m_C, 2H, CH_2), 7.33 (m, 1H, CH_{Ar}), 7.45 (m, 1H, CH_{Ar}), 7.64 (m, 1H, CH_{Ar}), 7.72 (m_C, 2H, CH_{Ar}), 10.34 (s, 1H, NH).
    4 δ (DMSO-d_6) = 1.89 (quin, $J = 7.5$ Hz, 2H, CH_2), 2.69 (t, $J = 7.2$ Hz, 2H, CH_2), 2.80 (t, $J = 7.2$ Hz, 2H, CH_2), 3.83 (s, 3H, O–CH_3), 6.92–8.09 (m, 7H, CH_{Ar}), 10.57 (s, 1H, NH).
    11 δ (CD_3OD) = 1.99 (quint, $J = 7.8$ Hz, 2H, CH_2), 2.85 (t, $J = 8.4$ Hz, 2H, CH_2), 2.91 (t, $J = 7.5$ Hz, 2H, CH_2), 7.31–7.39 (m, 3H, CH_{Ar}), 7.54–7.59 (m, 2H, CH_{Ar}), 7.66 (d, $J = 8.4$ Hz, 2H, CH_{Ar}).
    12 δ (CD_3OD) = 2.00 (quint, $J = 7.8$ Hz, 2H, CH_2), 2.84 (t, $J = 7.4$ Hz, 2H, CH_2), 2.94 (t, $J = 7.8$ Hz, 2H, CH_2), 7.12 (s, 1H, CH_{Ar}), 7.14 (s, 1H, CH_{Ar}), 7.37–7.42 (m, 3H, CH_{Ar}), 7.49–7.53 (m, 1H, CH_{Ar}).
    21 δ (DMSO-d_6) = 1.90–1.98 (ddd, $J = 7.55$ Hz, 2H, CH_2), 2.63–2.69 (dd, $J = 7.58$ Hz, 2H, CH_2), 2.76–2.81 (dd, $J = 7.48$ Hz, 2H, CH_2), 7.35–7.72 (m, 8H, CH_{Ar}), 10.36 (s, 1H, NH), 12.66 (s, 1H, OH).