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Biaryl analogues of teriflunomide as potent DHODH inhibitors

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ABSTRACT

The structure–activity relationships of a novel series of biaryl dihydroorotate dehydrogenase (DHODH) inhibitors related to teriflunomide are disclosed. These biaryl derivatives were the result of structure-based design and proved to be potent DHODH inhibitors which in addition showed good antiproliferative activities on peripheral blood mononuclear cells and good efficacies in vivo in the rat adjuvant-induced-arthritis model.

Dihydroorotate dehydrogenase (DHODH) is a mitochondrial enzyme that catalyzes the fourth step in the pyrimidine biosynthetic pathway, namely the conversion of dihydroorotate to orotate.[1] Inhibitors of DHODH exhibit anti-inflammatory and immunosuppressant properties and have demonstrated to be efficacious in the treatment of autoimmune diseases such as multiple sclerosis[2] and rheumatoid arthritis.[3]

Leflunomide **1** and brequinar **2** (Fig. 1) are two examples of low-molecular weight inhibitors of DHODH that have been in clinical development.[2–5] Leflunomide (Arava®, Sanofi-Aventis) is the only member of its class in the market. It is approved for the treatment of rheumatoid arthritis and psoriatic arthritis.[6] Teriflunomide **3** is the active metabolite of leflunomide[4] and is currently in Phase III clinical trials for multiple sclerosis.[7]

However, teriflunomide has a low potency against human DHODH[3] and a long half-life of approximately 2 weeks in plasma,[8] which could represent a serious obstacle for patients who need to withdraw the treatment in case of toxicity or pregnancy. Herein, we report our efforts to increase inhibitory potency against human DHODH enzyme and to adjust pharmacokinetic properties by reducing half-life.

Our strategy to develop DHODH inhibitors was based on the recently published co-crystal structure of the human enzyme with teriflunomide.[9] Analyses of this crystal (Fig. 2) provided a good starting point for structure-based design of more potent inhibitors.

The crystal revealed that trifluoromethyl phenyl group present in **3** only partially filled the binding cavity that is mainly lined by hydrophobic amino acid residues (Fig. 3).

Initially, methyl group of teriflunomide **3** was changed by ethyl and a two-fold increase in potency was observed. Then, in compound **4** (Fig. 4), where a bulkier hydrophobic group was introduced to better fill the cavity, an improvement in in vitro hDHODH activity by an order of magnitude was obtained (Table 1). In addition, the iv half-life of **4** was significantly reduced compared to **3**, but was still perceived as too long having in mind the different behaviour observed with teriflunomide between rat (14 h) and man (2 weeks) (Table 1).

The use of soft metabolic centres was then investigated as a mean to modulate and reduce $t_{1/2}$. The idea of introducing a methyl ester led to compound **5**. This molecule proved to be 15-fold more

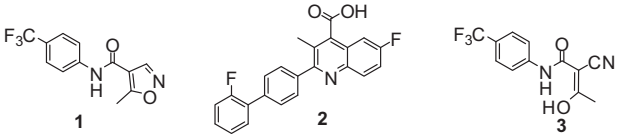

Figure 1. Reference compounds.

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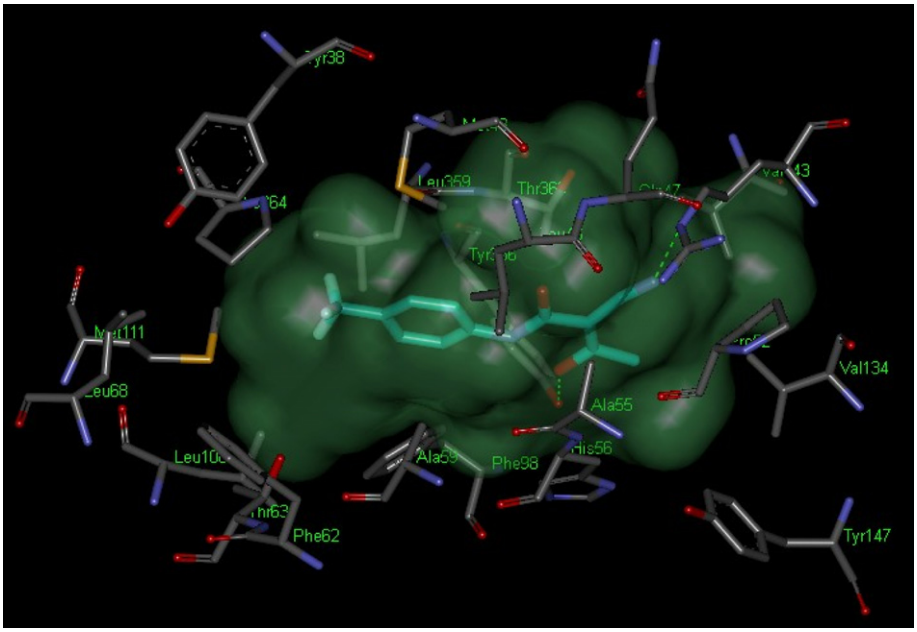


Figure 2. Crystal structure of teriflunomide in the lipophilic pocket of human DHODH (PDB code: 1D3H). The surface of the binding site is shown in green spheres. Dashed lines correspond to Hydrogen bond interactions with aminoacids Arg136 and Tyr356.

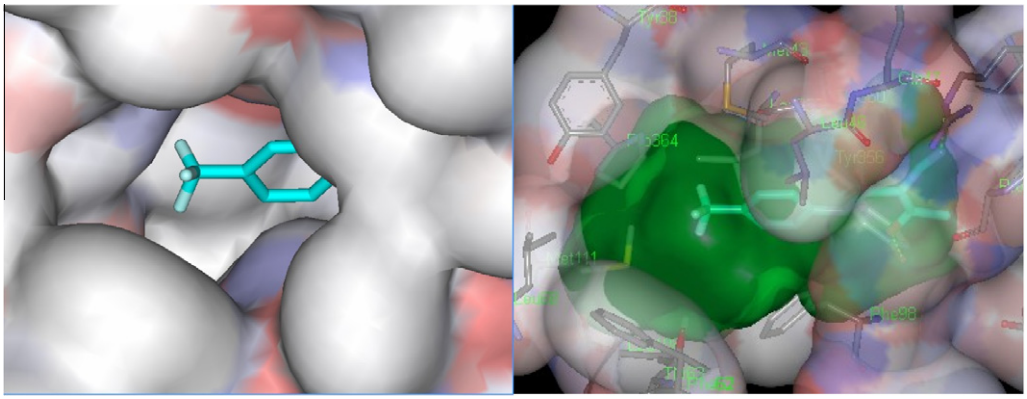


Figure 3. Surface representation of teriflunomide's trifluoromethylphenyl moiety inside the hydrophobic channel of human DHODH. The pictures show the space not filled by teriflunomide binding.

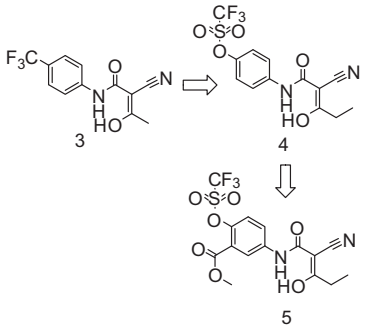


Figure 4. First teriflunomide analogues.

active than **3** in in vitro hDHODH activity and a greatly modified iv PK profile in rat (Table 1).

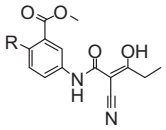
A concern with compound **5**, however, was that it contained a potentially displaceable triflate in the β-position of an α,β-unsaturated carboxylate which could be prone to promote covalent binding to proteins. The triflate group in **5** was put to good synthetic use, however, as a cross-coupling partner, which allowed a rapid assessment of SAR. Thus, introduction of a phenyl ring[10] was effected using a Suzuki reaction and variation of the substituents on this ring were explored with respect to both enzymatic and cellular immunosuppressive activity (Table 2).

A flexible synthetic route (Scheme 1) was employed in the synthesis of these series of compounds. Thus, synthesis of intermediate **7** was carried out by methylation of 2-hydroxy-5-nitrobenzoic acid **6**, subsequent reduction of the nitro group to give the corresponding aniline and coupling of this aniline with cyanoacetic acid. Intermediate **7** was treated with a triflating agent to give the

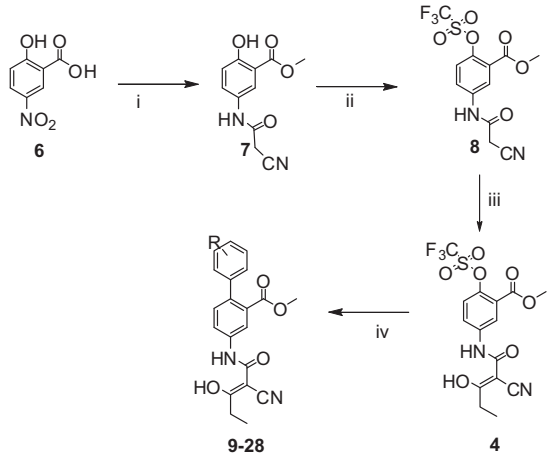
Table 1
Biological activities and pharmacokinetic profiles in rat

Compound	hDHODH IC_{50} (nM)	PK iv (1 mg/kg) $t_{1/2}$ (h)
3	1083	14.1
4	150	8
5	100	2

Table 2
Biological activities of biaryl derivatives



R	Compound	IC_{50} hDHODH (nM)
OSO_2CF_3	**5**	93
Phenyl	**9**	73
4-Fluorophenyl	**10**	110
2-Chlorophenyl	**11**	52
3-Chlorophenyl	**12**	31
4-Chlorophenyl	**13**	45
3-Trifluoromethoxyphenyl	**14**	27
3-Fluorophenyl	**15**	198
2,4-Difluorophenyl	**16**	31
3-Pyridine	**17**	40
4-Fluoro-2-methylphenyl	**18**	49
6-Fluoropyridin-3-yl	**19**	150
3,4-Difluorophenyl	**20**	97
3,4,5-Trifluorophenyl	**21**	137
4-Fluoro-2-methoxyphenyl	**22**	66
3-Ethoxyphenyl	**23**	59
1,3-Benzodioxol-5-yl	**24**	76
3-Methoxyphenyl	**25**	26
2,3-Dihydro-1,4-benzodioxin-6-yl	**26**	66
3-Difluoromethoxyphenyl	**27**	34
3-Cyclobutoxyphenyl	**28**	31



Scheme 1. Reagents and conditions: (i) (a) Me_2SO_4, acetone, reflux, 89%, (b) H_2, Ni-Ra, THF/MeOH, 40 psi, 85%, (c) $CNCH_2COOH$, EDC.HCl, rt, 89%; (ii) $PhN(OTf)_2$, Et_3N, THF, reflux, 58%; (iii) propionic anhydride, NaH, THF, rt, 78%; (iv) $RB(OH)_2$, $Pd(PPH_3)_4$, K_2CO_3, dioxane, 100 °C, 52–75%.

cyanoacetamide **8**, which was then acylated to afford the β-hydroxyenamide **4** using sodium hydride and propionic anhydride.

Finally, compounds **9–28** were synthesized by standard Suzuki coupling of compound **4** and the corresponding boronic acid or boronic acid pinacol ester employing tetrakis(triphenylphosphine)palladium(0) as a catalyst. Most boronic acids were either commercially available or prepared by standard literature methods.

This hydrophobic biphenyl tail[10] gave good binding affinity for human DHODH enzyme (IC_{50} <0.2 μM). As shown in Table 2, several compounds—for example, the 3-chloro (**12**, 31 nM) and 3-trifluoromethoxy (**14**; 27 nM) and closely related analogs—were particularly potent. Moreover, the attractive PK properties of the series were maintained by inclusion of the methyl ester, allowing us, in effect, to reduce the half-life (Table 4). The corresponding acid was found to be the main metabolite in human microsomes and it was 100-fold less active against hDHODH than the ester.

Table 3
In vitro profiles of selected derivatives

Compound	hDHODH IC_{50} (μM)	Proliferation hPBMC IC_{50} (μM)
3	1.08	46
10	0.11	2
12	0.03	34
13	0.04	13
14	0.03	37
16	0.03	6
21	0.14	19
24	0.08	18.5

Table 4
In vitro and in vivo profiles of selected derivatives

Compound	hDHODH IC_{50} (nM)	rDHODH IC_{50} (nM)	R/H Met%[a]	PK iv rat (1 mg/kg) $t_{1/2}$ (h)	AIA[b] ED_{50} (mg/Kg)
3	1083	26	0/3	14	2.88
10	110	350	19/17	0.9	49% @ 10 mg/kg

[a] Assay conditions: 1 mg/mL of microsomal protein in a phosphate buffer containing the compd at 5 μM at 37 °C. The reaction is initiated by the addition of the NADPH generating system. Results: % of compound degradation after 30 min incubation.

[b] Results are expressed as the inhibition of the area under the curve of the right hind paw swelling during the whole treatment period (10 days), using 6–7 rats per group. The arthritis was induced by sub-plantar injection of complete Freund's adjuvant in the left hind paw. Vehicle or compounds were dosed once daily in 0.5% methocel starting from day 10 post-induction and for 10 consecutive days.

In an attempt to identify the specific non-covalent binding interactions which were responsible for the high binding affinity, co-crystal structures with the human enzyme were determined for selected analogs. Herein we describe the structure of DHODH complex with the inhibitor **10**. The structure was solved at a resolution of 2.18 Å, and revealed the detailed binding mode of the ligand bound to the putative ubiquinone binding site.

The ligand forms two specific hydrogen bonds to DHODH, namely to the side chain residues Arg136 and Tyr356. Furthermore, binding of ligand is enhanced by a variety of hydrophobic interactions involving residues of the N-terminal helices α1 and α2—namely Tyr38, Met43, Leu46, Pro52, His56, Ala59, Phe62, Leu68 as well as Phe98, Met111, Thr360 and Pro364 (Fig. 5).

These hydrophobic interactions are consistent with the good activity of the lipophilic biphenyl derivatives (compounds **9**–**28**)—in particular, compounds **12**, **13**, 14 **16**, **17**, **25**, 27 and **28** with an IC_{50} <50 nM.

The co-crystal structure reveals that the newly introduced phenyl group fills the above hydrophobic binding cavity in a more complementary fashion than the CF_3 of **3** (Fig. 6).

Inhibition of DHODH at a cellular level translates into the inhibition of proliferation of mitogen-stimulated human peripheral blood mononuclear cells (hPBMCs),[11] which can be measured by incorporation of tritiated thymidine following 72 h of incubation of cells with phytohemagglutinin (PHA). Data for few selected compounds are presented in Table 3. Thus, enzymatic inhibition of hDHODH was translated into a good cellular effect, in particular compounds **10** and **16** which displayed potent antiproliferative activities on PBMCs.

The in vivo anti-inflammatory effect of compound **10** was further completed using the functional model of adjuvant induced arthritis (AIA) in rats[12] as shown in Table 4.

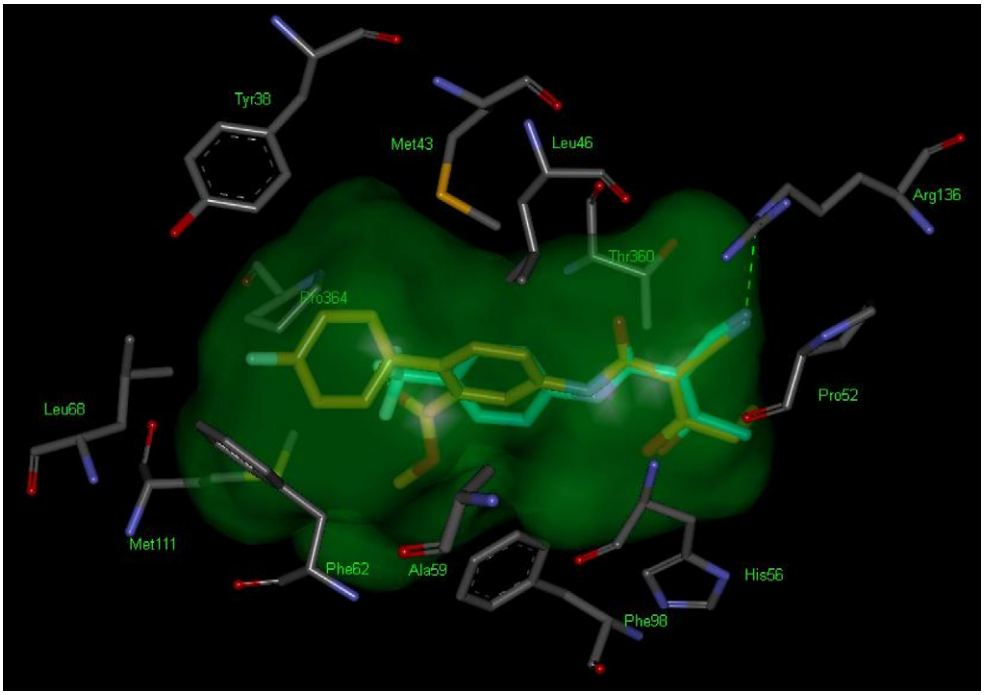


Figure 5. Superimposition of the co-crystallized complexes of teriflunomide (blue) and compound **10** (PDB code: 3U2O) (orange) at the binding site of human DHODH enzyme. The surface of the binding site is shown in green spheres. Dashed lines correspond to Hydrogen bond interactions with aminoacids Arg136 and Tyr356. The fluorophenyl group of compound **10** fills better the hydrophobic cavity of the enzyme and makes several interactions along the channel defined by residues: Tyr38, Met43, Leu46, Pro52, His56, Ala59, Phe62, Leu68, Phe98, Met111, Thr360 and Pro364.

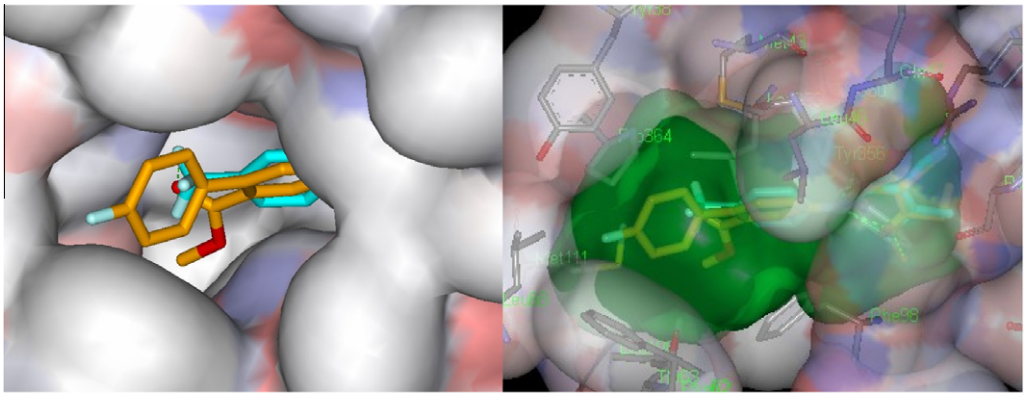


Figure 6. Surface representation of the hydrophobic channel of human DHODH. The picture shows the additional space filled by compound **10** (orange) in comparison with teriflunomide (blue) inside the hydrophobic cavity of the enzyme. Compound **10** makes more hydrophobic interactions inside the hydrophobic channel.

Compound **10** was shown to be active in the AIA model, although with a lower potency than teriflunomide. These results are not surprising in the light of the reduced half-life and the lower potency as rat DHODH inhibitor of compound **10** versus teriflunomide. Given that compound is 10 times more potent in the human enzyme, we would expect a similar efficacy as teriflunomide in humans and with a more acceptable pharmacokinetic profile.

A new family of potent and orally bioavailable hDHODH inhibitors has been designed, synthesized and characterized. Compound **10** is more than 20-fold more potent than teriflunomide as an antiproliferative in PBMCs and has an attractive PK profile in rat which is likely to obviate the liabilities (a half life of 2 weeks in man) of teriflunomide. Compound **10** has also shown efficacy in the experimental model of arthritis, AIA. Thus, compound **10** has been identified as an attractive starting point for further optimization. Further studies have addressed replacements for the β-hydroxyenamide moiety, to minimize the possible biphenylaniline metabolite that could be formed during Phase I metabolism. This work will be reported in due course.

Acknowledgment

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