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1 The role of fluorine in stabilizing the bioactive 2 conformation of dihydroorotate 3 dehydrogenase inhibitors

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10

11 **Abstract** Dihydroorotate dehydrogenase (DHODH) is an important drug target due to its
12 prominent role in the pyrimidine biosynthesis. Leflunomide and brequinar are two well-known
13 DHODH inhibitors, which bind to the enzyme in the same pocket with different binding modes.
14 We have recently realized a series of new inhibitors based on the 4-hydroxy-1,2,5-oxadiazole ring,
15 whose activity profile was found to be closely dependent on the degree of fluorine substitution at
16 the phenyl ring adjacent to the oxadiazole moiety; a positive influence of fluorine on the DHODH
17 inhibitory potency was previously observed by Baumgartner et al. PES scans showed that fluorine
18 has an important role in stabilizing the bioactive conformations; additionally fluorine influences
19 the balance between leflunomide-like and brequinar-like binding modes. These findings may serve
20 as a guide to design more potent DHODH inhibitors.

21

22 *Keywords: DHODH inhibitors; bioactive conformation; PES scan; fluorine;*
23 *strain energy.*

24

25 **Introduction**

26

27 Dihydroorotate dehydrogenase (DHODH) is a flavine-containing enzyme that
28 catalyzes the stereospecific conversion of (*S*)-dihydroorotate (DHO) to orotate
29 (ORO). Electrons resulting from this oxidation are transferred to ubiquinone
30 (CoQ) and finally to the cytochrome *c* oxidase of the respiratory chain [1]. Since
31 this transformation is the rate-limiting step of the *de novo* pyrimidine
32 biosynthesis, DHODH has become an appealing pharmaceutical target; its
33 inhibition leads to antiproliferative and immunomodulatory effects, which can be
34 exploited for the treatment of autoimmune diseases [2, 3]. The best known

35 DHODH inhibitors are leflunomide (**1**) and brequinar (**2**) (Fig. 1). The former is a
36 prodrug widely used in the treatment of rheumatoid arthritis; upon absorption, it
37 undergoes ring opening to its active metabolite A771726 (Fig. 1, **3**) [3]. The latter
38 was developed for cancer therapy and to combat the rejection of organ transplants,
39 but failed in clinical trials due to its narrow therapeutic window [4].
40 Previous crystallographic studies showed that DHODH has two distinct binding
41 sites: one for DHO/ORO and one for ubiquinone. Both A771726 and brequinar
42 bind the protein at the narrow end of the pocket used by ubiquinone to interact
43 with the reduced coenzyme: this channel contains lipophilic amino acids,
44 especially leucines and valines, and several polar residues such as Gln47, Arg136,
45 His56, Tyr356 and Thr360. The deprotonated enolic group of A771726 interacts
46 via hydrogen bonding with the phenolic moiety of Tyr356, while the amide
47 carbonyl forms a water-mediated hydrogen bond with Gln47 and Arg136 (Fig.
48 2a). The binding mode of brequinar is quite different: the carboxylate group forms
49 a salt bridge with Arg136 and a hydrogen bond with Gln47, while the biphenyl
50 moiety establishes a number of hydrophobic interactions with the lipophilic
51 residues of the channel (Fig. 2b) [5].
52 Our research group has recently explored the possibility of using the 1,2,5-
53 oxadiazole ring (furazan) as a bioisoster of the isoxazole moiety present in **1**.
54 Although these compounds undergo ring opening under physiological conditions
55 just as leflunomide, the resulting products proved to be very poor DHODH
56 inhibitors [6]. In order to improve their activity, the unsubstituted furazan moiety
57 was functionalized with a hydroxyl group. The hydroxyfurazan system, which is
58 stable under physiological conditions, should potentially maintain the correct
59 orientation of the deprotonated hydroxyl group, mimicking the enolic moiety of
60 A771726 [7]. In the attempt to validate this hypothesis, a docking simulation was
61 carried out using both rat and human enzymes. All these inhibitors appeared to
62 bind the protein in a brequinar-like fashion, with the deprotonated hydroxyl group
63 facing Arg136. However, using a different X-ray structure of human DHODH as
64 docking target, a 180° flip of the hydroxyfurazan moiety was observed, such that
65 the enolate group interacted with Tyr356 in a leflunomide-like fashion [7].
66 Marked variations of the ligand binding mode upon minor structural modifications
67 were observed also by Baumgartner and co-workers on another series of inhibitors
68 based on a fluorinated biphenyl scaffold [8]. In order to shed light on the

69 relationship between the structure of these inhibitors and the binding mode they
70 adopt in the DHODH pocket, we analyzed their complexes with the human
71 enzyme crystallized by Baumgartner (Table 1). These molecules bind the protein
72 in a leflunomide-like or brequinar-like fashion, and some of them show both
73 binding modes at once. The authors linked the in vitro activity data with the
74 prevailing mode of binding that the molecule adopts inside the DHODH pocket:
75 the more brequinar-like it is, the more active the inhibitor [8]. However, we
76 noticed that the inhibitory activity of these compounds is also related to their
77 substitution pattern, especially in the *ortho-ortho* ' positions of the central phenyl
78 ring. It is well known that flexible molecules do not bind the protein in their
79 lowest energy conformation [9]. The energy difference between the bioactive
80 conformation and the global minimum in solution configures a strain energy
81 penalty; its magnitude is inversely related to the activity of the molecule [9]. In
82 the attempt to investigate if these considerations hold true for DHODH, a
83 systematic conformational study was carried out on Baumgartner's series of
84 inhibitors, enhanced by two virtual models (**9** and **10**, Fig. 3) lacking fluorine
85 atoms on the central aromatic ring.

86

87 **Methods**

88

89 *Conformational search*

90

91 All molecules were modeled in their dissociated form, in accordance with their
92 pK_a values [6]. For compounds **4-8** (Table 1) crystallographic coordinates were
93 available [8], while 3D models **9** and **10** were built with the MOE modeling suite
94 [10], removing fluorine from compounds **4** and **7** respectively. A gas phase
95 optimization of all structures was carried out using the Newton-Raphson method
96 (MMFF94s force field, dielectric constant 4.0, no non-bonded cut-off) until the
97 gradient was lower than $0.05 \text{ kcal mol}^{-1} \text{ \AA}^{-1}$. In order to identify the most stable
98 geometries, a systematic conformational search was carried out by means of a
99 two-step procedure. In the first step the two torsional angles C3-N4-C5-C6 (ϕ)
100 and C7-C8-C11-C12 (ψ) (see Fig. 4) were varied over 10° increments, obtaining
101 1296 conformers. These structures underwent a constrained geometry
102 optimization blocking the two dihedrals at their initial values, while the rest of the

103 molecules was allowed to relax. A quantum-mechanical (QM) single-point DFT
104 calculation at the RB3LYP/6-31G(d) level of theory was run on the MMFF94s
105 minimum energy geometries, thus obtaining two potential energy surfaces (PES),
106 one purely MM and the other MM/QM. Once the local minima were identified
107 from the MM/QM PES, they were fully relaxed through a second unconstrained
108 DFT optimization carried out at the same level of theory. Once the stationary
109 points were characterized as true minima through a Hessian matrix calculation,
110 potential energies were refined through single-point calculations at the
111 RB3LYP/6-311G(2d, 2p) level. All QM calculations were performed using
112 FIREFLY [11]. Energy values for each structure were reported relative to the
113 global minimum.

114

115 *Docking simulation*

116

117 The starting conformations of **9** and **10** used for docking simulation were obtained
118 refining the MM local minima by an *ab initio* QM optimization at the
119 RHF/6-31G(d) level of theory using FIREFLY [11]. Atom-centred charges were
120 fit to the *ab initio* electrostatic potential through the RESP method [12].

121 The experimental crystallographic structures of DHODH complexes used as
122 docking targets were retrieved from the Protein Data Bank (PDB IDs 1D3G and
123 2BXV; resolutions 1.60 Å and 2.15 Å, respectively) [13]. Missing hydrogen
124 atoms were added in standard positions, then optimized using the SANDER
125 module of the AMBER 10 software package [14], while keeping heavy atoms
126 harmonically constrained to initial crystallographic coordinates with a force
127 constant of 32 kcal mol⁻¹ Å⁻². AMBER FF99 parameters and charges were
128 assigned to protein atoms, GAFF parameters coupled with QM-fitted RESP
129 charges [12] were used for the co-crystallized inhibitor and ORO, while values for
130 the FMN cofactor were taken from literature [15]. After removing the co-
131 crystallized inhibitor, docking of **9** and **10** was carried out using AutoDock 4.2
132 [16]. A 40×40×40 grid with 0.375 Å step size was centered on the inhibitors'
133 binding site and energy grid maps were pre-computed with AutoGrid, then
134 flexible docking was accomplished with AutoDock. The target proteins were kept
135 rigid, while ligands were left free to explore the conformational space inside the
136 DHODH cavity; 100 separate docking simulations were run on each protein using

137 the Lamarckian genetic algorithm with default parameters. This docking protocol
138 was able to closely reproduce the poses of the co-crystallized ligands present in
139 1D3G and 2BXV (RMSD 0.60 Å and 0.42 Å, respectively; Fig. S1, Electronic
140 supplementary material).

141

142 **Results and discussion**

143

144 MM PESs generated using the MMFF94s force field looked very similar among
145 each other; in particular, the fluorine atoms seemed not to exert any significant
146 effects on the conformational preferences of the molecules (Fig. 4a-b). These
147 results are in contrast with the well-known effect of fluorine atoms on aromatic
148 rings, especially when they occupy the *ortho-ortho* ' positions [17].

149 In contrast, MM/QM PESs were dramatically different from the purely MM ones
150 (Fig. 4c-d); most importantly, the QM method was able to put into evidence the
151 effect of fluorine substituents on the central phenyl ring, as expected. This effect
152 is indeed impressive, since the torsional angles which yielded minima on PESs of
153 non-fluorinated compounds correspond to maxima when fluorines are introduced
154 in the structures. In light of these considerations, only the MM/QM PESs will be
155 discussed further on.

156

157 *Non-fluorinated models*

158

159 PESs of models lacking aromatic fluorine atoms (Fig. 4c) showed two
160 symmetrical minima at 0 and 180° along the ϕ dihedral, while the ψ vs E curve is
161 characterized by two symmetric pairs of minima due to the presence of the *meta*
162 substituent in the distal phenyl ring; the same trend can be observed in all other
163 PESs. In global minimum conformations the amide group lies in the same plane as
164 the phenyl moiety (**9a** and **10a**, Table 2), allowing the formation of a charge-
165 enhanced hydrogen bond between the deprotonated carboxylic group and the
166 amide hydrogen.

167 To avoid biasing the outcome of our simulations towards either brequinar-like or
168 leflunomide-like poses, we decided to carry out docking of compounds **9** and **10**
169 on 1D3G and 2BXV protein templates, whose co-crystallized ligands show both
170 binding modes. As expected, the binding mode thus obtained was dependent on

171 the protein used as target, just as above described for the hydroxyfurazanyl
172 inhibitors. Both brequinar-like and leflunomide-like putative bioactive
173 conformations are tilted around ϕ , since the constraints imposed by the enzyme
174 cavity do not allow the amide group and the central phenyl ring to lie in the same
175 plane; however, the extent to which coplanarity is lost is quite different. In
176 leflunomide-like poses (**9b** and **10b**, Table 2) the amide portion is tilted by less
177 than 10° , making docked poses fairly superimposable to the global minima in gas
178 phase (Fig. S2a-b, Electronic supplementary material; RMSD 0.71 \AA and 0.50 \AA ,
179 respectively).

180 Moving to brequinar-like docked poses **9c** and **10c**, marked differences from
181 global minima are observed. Firstly, the charge-enhanced hydrogen bond found in
182 the leflunomide-like docked conformations is missing, probably due to an
183 underestimation of hydrogen bonding interactions in AutoDock's force field; as a
184 consequence, these structures are extremely unstable in gas phase ($+25 \text{ kcal mol}^{-1}$
185 for **9c** and $+19 \text{ kcal mol}^{-1}$ for **10c**). Moreover, the degree to which amide group
186 and phenyl ring are tilted compared to the QM global minima is much higher ($\phi =$
187 -44° and -24° , respectively), resulting in large RMSDs from the gas phase
188 conformations: 1.18 \AA and 0.99 \AA , respectively (Fig. S2c-d, Electronic
189 supplementary material). This indicates that brequinar-like bioactive poses are
190 very unlikely for these compounds, suggesting that in the absence of fluorine
191 atoms the leflunomide-like binding poses are largely favored.

192 Since the same considerations apply to the non-fluorinated inhibitors we recently
193 published [7], the low inhibitory activity of the latter may be reasonably attributed
194 to the prevalence of leflunomide-like poses, which according to Baumgartner
195 have a lower affinity for the DHODH pocket .

196

197 *Monofluorinated compounds*

198

199 The presence of a fluorine atom in the *ortho* position of the central phenyl ring
200 gives rise to three different minima depending on the ϕ torsional value (Fig. S3a,
201 Electronic supplementary material). For both the cyclopentene and the thiophene
202 derivatives, the most stable structures (**4a** and **7a**, Table 3) are characterized by
203 coplanarity of the amide group and the adjacent benzene ring, allowing for an
204 electrostatic interaction between the amide hydrogen and the aromatic fluorine.

205 The other, less stable local minima (**4b** and **7b**, Table 3) have quite different
206 geometries, in which the coplanarity between the amide group and the
207 *ortho*-fluorophenyl ring is lost together with the H \cdots F interaction, which is
208 replaced by the less favorable C=O \cdots F contact. Experimental bioactive poses
209 obtained via X-ray crystallography by Baumgartner and co-workers are also
210 reported in Table 3 for comparison. Both for **4** and **7** the leflunomide-like
211 conformations are more stable than the brequinar-like ones, which again accounts
212 for their relatively low activity. However, the energy gap between brequinar-like
213 and leflunomide-like poses is much higher for **4** (> 2 kcal mol $^{-1}$) than for **7** (0.72
214 kcal mol $^{-1}$); this explains why only in the case of **7** a fraction of the
215 experimentally determined complexes shows a brequinar-like binding mode.
216 Additionally, the conformational strain penalty to assume the leflunomide-like
217 binding mode is almost negligible for the thiophene derivative **7** (0.08 kcal mol $^{-1}$),
218 but not for the cyclopentene derivative **4** (1.24 kcal mol $^{-1}$). This finding justifies
219 the IC $_{50}$ value for compound **7** (44 nM) being one order of magnitude lower than
220 for **4** (280 nM).

221

222 *Difluorinated compounds*

223

224 Derivatives **5** and **8** are characterized by a fluorine atom in both the *ortho* and
225 *ortho'* positions of the central benzene ring. PESs contain sixteen almost
226 equivalent minima; minor energetic differences are only due to the long-range
227 interactions between the arylcarbamoyl moiety and the *meta* substituent on the
228 distal benzene ring (Fig. S3b, Electronic supplementary material). In contrast with
229 non-fluorinated and monofluorinated compounds, *ortho-ortho'* substituents force
230 the amide group to lie in a different plane with respect to the benzene ring, in
231 order to avoid steric and electrostatic clashes between the carbonyl oxygen and
232 the halogen. Potential energies of calculated and experimental conformations are
233 almost equivalent (Table 4): this suggests that likely both poses have similar
234 affinity for the DHODH pocket, the brequinar-like pose being slightly favored
235 (0.30 kcal mol $^{-1}$ above the global minimum for **5c** compared to 0.54 for the
236 leflunomide-like pose **5d**). All co-crystallized conformers are more closely
237 superimposable to the gas phase conformations than the monofluorinated
238 analogues (Fig. S2e-g, Electronic supplementary material, RMSD 0.30 Å, 0.44 Å

239 and 0.41Å, respectively); again, the lower conformational energy strain required
240 to assume the bioactive pose would account for their higher activity.

241

242 *Tetrafluorinated compound*

243

244 The only compound bearing four fluorine atoms published by Baumgartner *et al.*
245 is the cyclopentene derivative **6** (Table 1); its potential energy surface is similar to
246 the one of difluorinated inhibitors. The only remarkable difference is in the *E vs ψ*
247 profile, because the double *ortho-ortho*' substitution exerts its effect also on the
248 distal benzene ring, tilting it out of plane as observed for the amide group (Fig.
249 4d). Also in this case brequinar-like and leflunomide-like gas phase
250 conformations **6a** and **6b** are almost isoenergetic (Table 5). Similarly to the
251 difluorinated analogue, the crystallographic brequinar-like pose suffers a
252 moderately lower strain energy penalty than the leflunomide-like one, confirming
253 that fluorine has a beneficial effect in stabilizing the higher-affinity brequinar-like
254 binding mode.

255 In addition to the potential energy considerations discussed so far, it is reasonable
256 to expect that the higher rigidity imposed by the double *ortho-ortho*' substitution
257 pattern may favor binding also from an entropic point of view, since the loss of
258 conformational freedom upon binding will be incrementally lower moving from
259 tetra- to di-, mono-, and non-fluorinated analogues.

260 While the increasing degree of fluorination of the central benzene ring may
261 contribute improving interactions between the molecule and the hydrophobic
262 amino acids lining the DHODH cavity, especially leucines 46, 58 and 359, it
263 would be difficult to justify only on these bases the 100-fold increase in activity
264 observed in Baumgartner's series of inhibitors, particularly in the absence of
265 specific electrostatic or hydrogen bonding interactions.

266

267 **Summary**

268

269 Conformational preferences of a series of DHODH inhibitors were analyzed in
270 order to determine whether a correlation between their experimentally determined
271 binding mode and their affinity could be found. The MMFF94s force-field failed
272 to properly address *ortho-ortho*' effects; therefore, a systematic conformational

273 scan was carried out with a DFT method, in order to obtain MM/QM potential
274 energy surfaces of higher quality. Analysis of the latter allowed establishing a
275 clear link between the degree of fluorine substitution, the preferred binding mode
276 and the inhibitory activity. Translating these observations to the non-fluorinated
277 models **9** and **10**, we were able to find a sound justification of the low activity of a
278 series of inhibitors we realized in the recent past, which shared a scaffold largely
279 reminiscent of Baumgartner's compounds but lacked fluorine substituents. Our
280 conformational analysis also underlined the role of incremental fluorine
281 substitution in stabilizing the brequinar-like binding mode, which has been
282 previously found to be connected with higher inhibitory potency. Our work sheds
283 light on the molecular determinants which lead to effective DHODH inhibition,
284 and may serve as a guide to design more potent analogues by molecular modeling
285 techniques.

286

287 **Acknowledgments**

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289

290 **References**

291

- 292 1. Leban J, Vitt D (2011) Human dihydroorotate dehydrogenase inhibitors, a novel approach for
293 the treatment of autoimmune and inflammatory diseases. *Arzneim-Forsch* 61:66-72. doi
294 10.1055/s-0031-1296169 and references cited therein
- 295 2. Batt DG (1999) Inhibitors of dihydroorotate dehydrogenase. *Exp Opin Ther Patents* 9:41-45.
296 doi 10.1517/13543776.9.1.41
- 297 3. Breedveld FC, Dayer JM (2000) Leflunomide: mode of action in the treatment of rheumatoid
298 arthritis. *Ann Rheum Dis* 59:841-849. doi 10.1136/ard.59.11.841
- 299 4. Pally C, Smith D, Jaffee B, Magolda R, Zehender H, Dorobek B, Donatsch P, Papageorgiou
300 C, Schuurman HJ (1998) Side effects of brequinar and brequinar analogues, in combinations
301 with cyclosporine, in the rat. *Toxicology* 127:207-222. doi 10.1016/S0300-483X(98)00026-2
- 302 5. Liu S, Neidhardt EA, Grossman TH, Ocain T, Clardy J (2000) Structure of human
303 dihydroorotate dehydrogenase in complex with antiproliferative agents. *Structure* 8:25-33. doi
304 10.1016/S0969-2126(00)00077-0
- 305 6. Giorgis M, Lolli ML, Rolando B, Rao A, Tosco P, Chaurasia S, Marabello D, Fruttero R,
306 Gasco A (2011) 1,2,5-oxadiazole analogues of leflunomide and related compounds. *Eur J*
307 *Med Chem* 46:383-392. doi 10.1016/j.ejmech.2010.10.029

- 308 7. Lolli ML, Giorgis M, Tosco P, Foti A, Fruttero R, Gasco A (2012) New inhibitors of
309 dihydroorotate dehydrogenase (DHODH) based on the 4-hydroxy-1,2,5-oxadiazol-3-yl
310 (hydroxyfurazanyl) scaffold. *Eur J Med Chem* 49:102-109. doi 10.1016/j.ejmech.2011.12.038
- 311 8. Baumgartner R, Walloschek M, Kralik M, Gotschlich A, Tasler S, Mies J, Leban J (2006)
312 Dual binding mode of a novel series of DHODH inhibitors. *J Med Chem* 49:1239-1247. doi
313 10.1021/jm0506975
- 314 9. Perola E, Charifson PS (2004) Conformational analysis of drug-like molecules bound to
315 proteins: an extensive study of ligand reorganization upon binding. *J Med Chem*
316 47:2499-2510. doi 10.1021/jm030563w
- 317 10. Molecular Operating Environment version 2010.10 (MOE). Chemical Computing Group Inc.,
318 Montréal, Québec, Canada, 2010
- 319 11. Granovsky AA, Firefly version 7.1.G, <http://classic.chem.msu.su/gran/firefly/index.html>.
320 Accessed 30 September 2012
- 321 12. Wang J, Cieplak P, Kollman PA (2000) How well does a restrained electrostatic potential
322 (RESP) model perform in calculating conformational energies of organic and biological
323 molecules? *J Comput Chem* 21:1049-1074. doi 10.1002/1096-
324 987X(200009)21:12<1049::AID-JCC3>3.0.CO;2-F
- 325 13. The RCSB Protein Data Bank. <http://www.rcsb.org/>. Accessed 30 September 2012
- 326 14. Case DA, Darden TA, Cheatham III TE, Simmerling CL, Wang J, Duke RE, Luo R, Crowley
327 M, Walker RC, Zhang W, Merz KM, Wang B, Hayik S, Roitberg A, Seabra G, Kolossvary I,
328 Wong KF, Paesani F, Vanicek J, Wu X, Brozell SR, Steinbrecher T, Gohlke H, Yang L, Tan
329 C, Mongan J, Hornak V, Cui G, Mathews DH, Seetin MG, Sagui C, Babin V, Kollman PA
330 (2008) AMBER 10. University of California, San Francisco (USA)
- 331 15. Schneider C, Sühnel J (1999) A molecular dynamics simulation of the flavin mononucleotide-
332 RNA aptamer complex. *Biopolymers* 50:287-302. doi 10.1002/(SICI)1097-
333 0282(199909)50:3<287::AID-BIP5>3.0.CO;2-G
- 334 16. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ (1998)
335 Automated docking using Lamarckian genetic algorithm and an empirical binding free energy
336 function. *J Comput Chem* 19:1639-1662. doi 10.1002/(SICI)1096-
337 987X(19981115)19:14<1639::AID-JCC10>3.0.CO;2-B
- 338 17. Purser S, Moore PR, Swallow S, Gouverneur V (2008) Fluorine in medicinal chemistry.
339 *Chem Soc Rev* 37:320-330. doi 10.1039/B610213C
- 340

341 **Figure captions**

342

343 **Fig. 1** Structures of leflunomide (**1**), its active metabolite A771726 (**3**), and brequinar (**2**)

344

345 **Fig. 2** Binding mode of A771726 (a) and a close analogue of brequinar (b) inside DHODH (PDB
346 IDs 1D3H and 1D3G, respectively)

347

348 **Fig. 3** Virtual models lacking aromatic fluorine atoms added to Baumgartner's series

349

350 **Fig. 4** MM potential energy surfaces for compounds **9** (a) and **6** (b) and their respective MM/QM
351 curves (c and d). The potential energy values relative to the global minimum (kcal mol^{-1}) are
352 reported on the z axis vs the torsional angles ϕ and ψ (values expressed in degrees)