# Dihydroorotate Dehydrogenase Inhibitors: Quantitative Structure-activity Relationship Analysis

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***Purpose.*** The main purpose of this study is to analyze the quantitative structure-activity relationship of two series of dihydroorotate dehydrogenase inhibitors (leflunomide and quinoline carboxylic acid analogues), and to determine the structural requirements for optimum activity of these analogues.

***Methods.*** A new CQSAR program was used in deriving regression equations and calculating the octanol/water partition coefficient and the molar refractivity values. The molecular modeling was performed using the HyperChem® program.

***Results.*** Statistically significant correlations were obtained using a combination of 3–4 parameters. The structural requirements for optimum activity and critical regions for the inhibitory activity of dihydroorotate dehydrogenase were identified.

***Conclusions.*** The quantitative structure-activity relationship analysis demonstrated that two series of dihydroorotate dehydrogenase inhibitors may bind to different binding sites on the enzyme. These results provide a better understanding of dihydroorotate dehydrogenase inhibitor-enzyme interactions, and may be useful for further modification and improvement of inhibitors of this important enzyme.

**KEY WORDS:** brequinar sodium; CQSAR; dihydroorotate dehydrogenase (DHODH); leflunomide; quantitative structure-activity relationship (QSAR); quinoline carboxylic acid.

## INTRODUCTION

Dihydroorotate dehydrogenase (DHODH), a rate-limiting enzyme of *de novo* pyrimidine biosynthesis, catalyzes the oxidation of dihydroorotate to orotic acid (1). Orotate reacts with phosphoribosylpyrophosphate (PRPP) to form orotidylate (a pyrimidine nucleotide), then decarboxylated to yield uridylate (UMP), a major pyrimidine nucleotide needed for DNA synthesis (2). The activity of DHODH has been found to decrease in hepatocarcinoma *in vitro* (3) and during hepatocarcinogenesis *in situ* (4). This enzyme may serve as a target for developing therapeutic agents for the treatment of cancer, malaria, rheumatoid arthritis, and possibly other immune disorders.

A number of DHODH inhibitors have been shown by various investigators to have anticancer, antimalarial and antirheumatic activities (5–7). A recent anticancer drug candidate brequinar sodium [Dup 785, NSC 368390, 6-fluoro-2-(2′-fluoro-1,1′-biphenyl-4-yl)-3-methyl-4-quinoline carboxylic acid sodium (**A**), Figure 1 A], a quinoline carboxylic acid analogue, exerts its antitumor activity by inhibiting the activity of DHODH (6). In a recent study (7), a series of analogues of the active metabolite (**C**) (Figure 1 C) of an immunosuppressive agent leflunomide (**B**) (Figure 1 B) have been synthesized and found to inhibit DHODH. From this series, one compound, HR 325 (**D**) (Figure 1 D) has progressed into phase II clinical trials for the treatment of rheumatoid arthritis. Chen (6) and Kuo (7) have reported the structure-activity relationships (SAR) of these two series of analogues, respectively. The purpose of this report is to analyze the quantitative structure-activity relationship (QSAR) of the analogues of the active metabolite of leflunomide and the quinoline carboxylic acid analogues, and to determine the structural requirements of these two different series of analogues for optimum activity. The QSAR together with the modeling studies will provide a more precise elucidation of the molecular forces involved in the DHODH inhibitor-enzyme interactions.

## METHODS

The biological activities of analogues of the active metabolite of leflunomide and the quinoline carboxylic acid analogues were taken from the papers by Kuo *et al.* (7) and Chen *et al.* (6) respectively. Not every compound from Kuo's paper was included in the QSAR analysis because of the lack of parameters (6 compounds) and the exact $IC_{50}$ values ($IC_{50} > 10^5$ nM for 6 compounds). One pair of geometric isomers was also excluded from the regression analysis due to the single isomer pair (8). Each series of analogues was subdivided into two or three subgroups according to the substituents at different positions. Sigma para values ($\sigma_p$), molar refractivity (MR) and hydrophobic constant ($\pi$) of substituents were obtained from the CQSAR program (9). The calculated *n*-octanol/water partition coefficient (Clog P) and the calculated molar refractivity (CMR) of the whole molecules were automatically calculated after the parent structures and substituent structures were entered via SMILES using the CQSAR program. All regression equations were derived with the CQSAR program using the permutation of different physicochemical parameters. The space-filled models of HR 325 and brequinar sodium were obtained after global geometry optimization and energy minimization using the HyperChem® MM+ force field method (10). The molecular dipole moments for the unionized forms were calculated using the AM1 method.

## RESULTS AND DISCUSSIONS

### Analogues of the Active Metabolite of Leflunomide

The analogues of the active metabolite of leflunomide were classified into two subgroups. One is aromatic substituted subgroup, and the second is the side chain 3-substituted subgroup (see Tables I and III for the structures). The bioactivities and the physicochemical parameters used in the regression analysis of the aromatic substituted subgroup are summarized in Table I. The results of stepwise regression analysis are given in equations 1-14. I is an indicator variable representing individually the presence (I = 1) or absence (I = 0) of electron-withdrawing group at meta-position, or the presence (I = 1) or absence (I = 0) of ortho-substituents. Among various parameters ($\sigma_p$, $\sigma_m$, $\sigma_o$, $\pi$, hydrogen bonds (Hb) and log MWx of substituents, and Clog P, CMR and the

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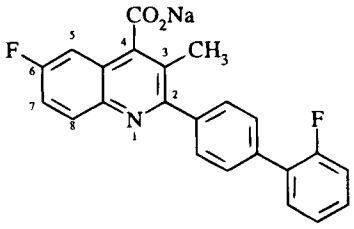
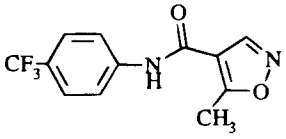
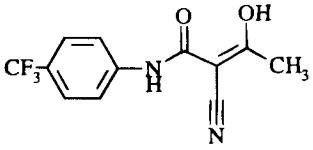


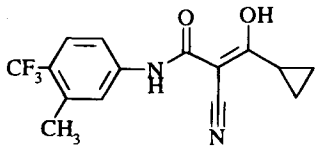
A. Brequinar sodium



B. Leflunomide



C. The active metabolite of leflunomide



D. HR 325

**Fig. 1.** The structures of different DHODH inhibitors.

calculated dipole moment (μ) of the whole molecule), Clog P, CMR, $\sigma_p$ and I make the most important contributions to the activity against DHODH. An $r$ of 0.873 and 0.834 was obtained without deleting any outliers for the data obtained in mouse and rat DHODH, respectively (see equations 4 and 11). Deletion of three outliers in equations 4 and 11 resulted in a better correlation with an $r$ of 0.910 and 0.901 for mouse and rat enzymes, respectively (see equations 5 and 12). Clog P and $\sigma_p$ always make positive contributions, while CMR and I make negative contributions to the activity in enzymes from both species, suggesting that unfavorable contributions of introducing an ortho-substituent or an electron-withdrawing meta-substituent. This may be due to the steric effects of the ortho-substituent and electronic effects of meta-substituent. Furthermore, electron-withdrawing groups at para-position enhance the activity. An appropriate substituent with a larger Clog P and a smaller CMR for the whole molecule also favor the activity.

By adding a $(\text{Clog P})^2$ term in the regression analysis, equations 6 and 13 were obtained with an improved $r$ and a decreased $s$. As shown by a larger 95% confidence level and a stepwise $F$-test of the $(\text{Clog P})^2$ term ($F_{1,34} = 1.36 < F_{1,34\ .95} = 4.17$ for the $(\text{Clog P})^2$ term in equation 6, $F_{1,36} = 3.47 < F_{1,36\ .95} = 4.08$ for the $(\text{Clog P})^2$ term in equation 13), equations 6 and 13 are not better than equation 5 and 12. By dividing the whole data into two subclasses according to the substituents at para-position, namely aliphatic substituted subclass (compounds 1-35 in Table I) and aromatic substituted subclass (compounds 36-42 in Table I), a much better correlation was obtained for the aliphatic substituted subclass in both mouse and rat enzymes (see equations 7 and 14), while no significant correlation could be found for the aromatic substituted subclass (not shown) due to the limited data points. $IC_{50}$ values for the aromatic substituted subclass are generally greater than 500 nM except one compound with a cis *p*-chlorophenylethenyl group (7), suggesting that the aromatic substituent at para-position reduces the inhibitory activity against DHODH. The squared correlation matrix of the parameters used in the regression analysis of the aromatic substituted subgroup is shown in Table II. From the $r^2$ values, it was found that some covariance existed between Clog P and CMR ($r^2 = 0.398$), the other parameters were not interdependent.

*Mouse DHODH*

$$\text{Log } 1/IC_{50} = 0.426\,(\pm 0.262)\,\text{Clog P} + 5.281(\pm 0.654)$$
$$n = 40\ r = 0.459\ s = 0.836\ F_{1,38} = 10.16\ p < 0.01 \quad (1)$$

$$\text{Log } 1/IC_{50} = 0.840\,(\pm 0.266)\,\text{Clog P} - 0.730(\pm 0.294)\,\text{CMR} + 9.980\ (\pm 2.016)$$
$$n = 40\ r = 0.721\ s = 0.661\ F_{2,37} = 19.99\ p < 0.01 \quad (2)$$

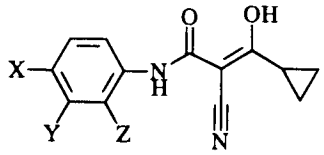
$$\text{Log } 1/IC_{50} = 0.906\,(\pm 0.221)\,\text{Clog P} - 0.881\ (\pm 0.251)\,\text{CMR} - 1.289\ (\pm 0.585)\,\text{I} + 11.128\ (\pm 1.781)$$
$$n = 40\ \ r = 0.827\ \ s = 0.544\ \ F_{3,36} = 25.90\ \ p < 0.01 \quad (3)$$

$$\text{Log } 1/IC_{50} = 0.872\,(\pm 0.195)\,\text{Clog P} - 0.828\ (\pm 0.223)\,\text{CMR} - 1.066\ (\pm 0.529)\,\text{I} + 0.810\ (\pm 0.463)\,\sigma_p + 10.553\ (\pm 1.673)$$
$$n = 40\ \ r = 0.873\ \ s = 0.478\ \ F_{4,35} = 28.10\ \ p < 0.01 \quad (4)$$

$$\underline{\text{Log } 1/IC_{50} = 0.912\,(\pm 0.163)\,\text{Clog P} - 0.776\,(\pm 0.187)\,\text{CMR} - 1.050\ (\pm 0.442)\,\text{I} + 0.575\ (\pm 0.402)\,\sigma_p + 10.103\ (\pm 1.423)}$$
$$\underline{n = 37\ r = 0.910\ s = 0.398\ F_{4,32} = 38.55\ p < 0.01} \quad (5)$$

$$\text{Log } 1/IC_{50} = 1.274\,(\pm 0.702)\,\text{Clog P} - 0.119\ (\pm 0.200)\,(\text{Clog P})^2 - 0.706\ (\pm 0.302)\,\text{CMR} - 1.114\ (\pm 0.533)\,\text{I} + 0.744\ (\pm 0.474)\,\sigma_p + 9.446\ (\pm 2.600)\,\text{Clog P}_o = 5.353$$
$$n = 40\ r = 0.879\ s = 0.475\ F_{5,34} = 23.02\ p < 0.01 \quad (6)$$

**Table I.** The Enzyme Inhibitory Activities and the Physicochemical Parameters Used in the Regression Analysis of Aromatic Substituted Analogues



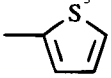
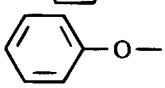
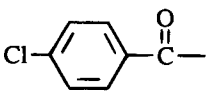
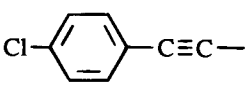
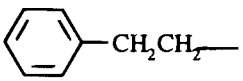
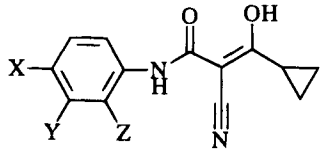
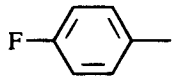
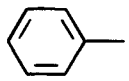
| Compd No. | X | Y | Z | Mouse DHODH $IC_{50}$[a] (nM) | Mouse DHODH log 1/$IC_{50}$ (M) obsd. | Mouse DHODH log 1/$IC_{50}$ (M) calcd.[b] | Rat DHODH $IC_{50}$[a] (nM) | Rat DHODH log 1/$IC_{50}$ (M) obsd. | Rat DHODH log 1/$IC_{50}$ (M) calcd.[c] | Clog P[d] | CMR[d] | $\sigma_p$[e] | I[f] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | H | H | H | 1580 | 5.80 | 6.22 | 2000 | 5.70 | 6.74 | 1.21 | 6.42 | 0.00 | 0 |
| 2 | $CH_3$ | H | H | 288 | 6.54 | 6.22 | 234 | 6.63 | 6.41 | 1.71 | 6.88 | –0.17 | 0 |
| 3 | $CF_3$ | H | H | 47 | 7.33 | 7.35 | 21 | 7.68 | 8.23 | 2.54 | 6.93 | 0.54 | 0 |
| 4 | H | $CF_3$ | H | 525 | 6.28 | 5.99 | 479 | 6.32 | 6.23 | 2.54 | 6.93 | 0.00 | 1 |
| 5 | Cl | H | H | 36 | 7.44 | 6.86 | 63 | 7.20 | 7.43 | 2.18 | 6.91 | 0.23 | 0 |
| 6 | H | Cl | H | 3000 | 5.52 | 5.68 | 343 | 6.46 | 5.98 | 2.18 | 6.91 | 0.00 | 1 |
| 7 | H | H | Cl | 16600 | 4.78 | 4.90 | 13300 | 4.88 | 5.37 | 1.33 | 6.91 | 0.00 | 1 |
| 8 | Br | H | H | 36 | 7.44 | 6.77 | 79 | 7.10 | 7.29 | 2.33 | 7.20 | 0.23 | 0 |
| 9 | CN | H | H | 42 | 7.38[h] | (6.26) | 53 | 7.28 | 7.52 | 1.24 | 6.90 | 0.66 | 0 |
| 10 | $-CH_2CN$ | H | H | 37200 | 4.43 | 5.07 | 4570 | 5.34 | 5.83 | 0.63 | 7.36 | 0.18 | 0 |
| 11 | $CF_3S$ | H | H | 100 | 7.00 | 7.00 | 5 | 8.30 | 7.70 | 2.87 | 7.74 | 0.50 | 0 |
| 12 | $CF_3SO$ | H | H | 417 | 6.38 | 6.58 | 16 | 7.80 | 7.60 | 2.32 | 7.77 | 0.69 | 0 |
| 13 | $CF_3SO_2$ | H | H | 89 | 7.05 | 6.72 | 3 | 8.52 | 8.06 | 2.32 | 7.80 | 0.96 | 0 |
| 14 | $CH_3S$ | H | H | 445 | 6.35 | 5.75 | 13 | 7.89[h] | (6.06) | 1.77 | 7.69 | 0.00 | 0 |
| 15 | $CH_3SO$ | H | H | 22400 | 4.65 | 5.58 | 832 | 6.08 | 5.77 | 0.21 | 7.72 | 0.49 | 0 |
| 16 | $CH_3SO_2$ | H | H | 15100 | 4.82 | 4.69 | 158 | 6.80 | 6.14 | 0.21 | 7.76 | 0.72 | 0 |
| 17 | $CF_3O$ | H | H | 173 | 6.76 | 6.99 | 5 | 8.30 | 7.65 | 2.39 | 7.08 | 0.35 | 0 |
| 18 | $CH_3O$ | H | H | 3720 | 5.43 | 5.65 | 186 | 6.73 | 5.78 | 1.28 | 7.04 | –0.27 | 0 |
| 19 | OH | H | H | —[g] | —[g] | — | 7940 | 5.10 | 5.47 | 0.54 | 6.57 | –0.37 | 0 |
| 20 | $NO_2$ | H | H | 50 | 7.30 | 6.46 | 21 | 7.68 | 7.81 | 1.50 | 7.03 | 0.78 | 0 |
| 21 | $H_2N$ | H | H | 31600 | 4.50 | 4.37 | 21900 | 4.66 | 4.29 | –0.02 | 6.88 | –0.66 | 0 |
| 22 | $CH_3CO$ | H | H | 3800 | 5.42 | 5.67 | 68 | 7.17 | 6.73 | 1.11 | 7.38 | 0.50 | 0 |
| 23 | $H_2NCO$ | H | H | —[g] | —[g] | — | 14100 | 4.85 | 5.76 | 0.01 | 7.29 | 0.36 | 0 |
| 24 | HOOC- | H | H | 3720 | 5.43 | 6.00 | 1480 | 5.83[h] | (7.00) | 1.24 | 7.07 | 0.45 | 0 |
| 25 | $CH_3O_2C-$ | H | H | 1120 | 5.95 | 6.00 | 158 | 6.80 | 6.89 | 1.64 | 7.54 | 0.45 | 0 |
| 26 | $CF_3$ | $CH_3$ | H | 55 | 7.26 | 7.45 | 14 | 7.85 | 8.19 | 3.04 | 7.39 | 0.54 | 0 |
| 27 | $CF_3$ | $C_2H_5$ | H | 71 | 7.15 | 7.57 | 40 | 7.40 | 8.18 | 3.57 | 7.86 | 0.54 | 0 |
| 28 | $C_2F_5$ | $CH_3$ | H | 282 | 6.55 | 6.93 | 11 | 7.96 | 7.64 | 2.91 | 7.89 | 0.52 | 0 |
| 29 | Cl | $CH_3$ | H | 40 | 7.40 | 6.95 | 33 | 7.48 | 7.39 | 2.68 | 7.38 | 0.23 | 0 |
| 30 | Cl | H | $CH_3$ | 3720 | 5.43 | 5.44 | 1250 | 5.90 | 5.99 | 2.18 | 7.38 | 0.23 | 1 |
| 31 | $CH_3$ | Cl | H | 282 | 6.55 | 6.72 | 79 | 7.10 | 6.69 | 2.68 | 7.38 | –0.17 | 1 |
| 32 | Br | $CH_3$ | H | 63 | 7.20 | 6.87 | 50 | 7.30 | 7.26 | 2.83 | 7.66 | 0.23 | 0 |
| 33 | CN | $CH_3$ | H | 36 | 7.44[h] | (6.49) | 28 | 7.55 | 7.59 | 1.88 | 7.36 | 0.66 | 0 |
| 34 | $CF_3S$ | $CH_3$ | H | 178 | 6.75 | 7.10 | 11 | 7.96 | 7.67 | 3.37 | 8.20 | 0.50 | 0 |
| 35 | $CF_3O$ | $CH_3$ | H | 178 | 6.75 | 7.08 | 18 | 7.74 | 7.61 | 2.89 | 7.55 | 0.35 | 0 |
| 36 |  | H | H | 2510 | 5.60 | 6.07 | 631 | 6.20 | 6.14 | 2.99 | 8.74 | 0.05 | 0 |
| 37 |  | H | H | 630 | 6.20 | 6.19 | 295 | 6.53 | 6.04 | 3.46 | 9.08 | –0.03 | 0 |
| 38 |  | H | H | 562 | 6.25 | 5.82 | 590 | 6.23 | 6.11 | 3.50 | 9.92 | 0.40 | 0 |
| 39 |  | H | H | 5000 | 5.30 | 5.61 | 17800 | 4.75 | 5.50 | 3.76 | 10.30 | 0.13 | 0 |
| 40 |  | H | H | 630 | 6.20 | 5.71 | 178 | 6.75[h] | (5.35) | 3.65 | 9.86 | –0.12 | 0 |

**Table I.** *(continued)*



| Compd No. | X | Y | Z | Mouse DHODH $IC_{50}$[a] (nM) | Mouse DHODH log $1/IC_{50}$ (M) obsd. | Mouse DHODH log $1/IC_{50}$ (M) calcd.[b] | Rat DHODH $IC_{50}$[a] (nM) | Rat DHODH log $1/IC_{50}$ (M) obsd. | Rat DHODH log $1/IC_{50}$ (M) calcd.[c] | Clog P[d] | CMR[d] | $\sigma_p$[e] | I[f] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 41 |  | H | H | 8510 | 5.07[h] | (6.19) | 2140 | 5.67 | 6.19 | 3.29 | 8.95 | 0.06 | 0 |
| 42 |  | H | H | 1580 | 5.80 | 5.98 | 1480 | 5.83 | 5.94 | 3.09 | 8.93 | –0.01 | 0 |

[a] From ref. 7.
[b] Calculated from equation 5.
[c] Calculated from equation 12.
[d] Calculated values using the CQSAR program.
[e] Obtained from the CQSAR program.
[f] For compounds with electron-withdrawing substituents at meta-position or with substituents at ortho-position, I = 1; for other compounds, I = 0.
[g] Compounds with an $IC_{50}$ of greater $10^5$ nM, not included in the regression analysis.
[h] Statistical outliers, not included in equations 5 and 12.

$$\text{Log } 1/IC_{50} = 0.861\ (\pm 0.198)\ \text{Clog P} - 0.975\ (\pm 0.505)\ \text{CMR} - 0.423\ (\pm 0.232)\ \text{I} + 0.917\ (\pm 0.572)\ \sigma_p + 11.605\ (\pm 3.713)$$
$$n = 33\ r = 0.886\ s = 0.486\ F_{4,28} = 25.35\ p < 0.01 \quad (7)$$

*Rat DHODH*

$$\text{Log } 1/IC_{50} = 1.826\ (\pm 0.782)\ \sigma_p + 6.279\ (\pm 0.337)$$
$$n = 42\ r = 0.587\ s = 0.871\ F_{1,40} = 20.95\ p < 0.01 \quad (8)$$

$$\text{Log } 1/IC_{50} = 1.747\ (\pm 0.726)\ \sigma_p + 0.328\ (\pm 0.232)\ \text{Clog P} + 5.604\ (\pm 0.586)$$
$$n = 42\ r = 0.672\ s = 0.807\ F_{2,39} = 16.04\ p < 0.01 \quad (9)$$

$$\text{Log } 1/IC_{50} = 1.693\ (\pm 0.617)\ \sigma_p + 0.657\ (\pm 0.254)\ \text{Clog P} - 0.613\ (\pm 0.299)\ \text{CMR} + 9.612\ (\pm 2.076)$$
$$n = 42\ r = 0.784\ s = 0.684\ F_{3,38} = 20.23\ p < 0.01 \quad (10)$$

**Table II.** The Squared Correlation Matrix ($r^2$) of the Parameters Used in the Regression Analysis of the Aromatic Substituted Analogues ($n = 42$)

| | Clog P | CMR | $\sigma_p$ | I |
|---|---|---|---|---|
| Clog P | 1.000 | | | |
| CMR | 0.398 | 1.000 | | |
| $\sigma_p$ | 0.006 | 0.000 | 1.000 | |
| I | 0.000 | 0.051 | 0.036 | 1.000 |

$$\text{Log } 1/IC_{50} = 1.503\ (\pm 0.569)\ \sigma_p + 0.726\ (\pm 0.233)\ \text{Clog P} - 0.743\ (\pm 0.282)\ \text{CMR} - 1.077\ (\pm 0.677)\ \text{I} + 10.612\ (\pm 2.113)$$
$$n = 42\ r = 0.834\ s = 0.617\ F_{4,37} = 21.09\ p < 0.01 \quad (11)$$

$$\text{Log } 1/IC_{50} = 1.769\ (\pm 0.470)\ \sigma_p + 0.725\ (\pm 0.188)\ \text{Clog P} - 0.856\ (\pm 0.237)\ \text{CMR} - 1.043\ (\pm 0.544)\ \text{I} + 11.358\ (\pm 1.845)$$
$$n = 39\ r = 0.901\ s = 0.494\ F_{4,34} = 36.62\ p < 0.01 \quad (12)$$

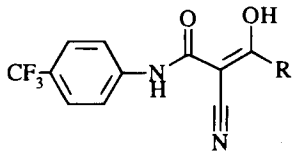
$$\text{Log } 1/IC_{50} = 1.372\ (\pm 0.568)\ \sigma_p + 1.440\ (\pm 0.785)\ \text{Clog P} - 0.222\ (\pm 0.233)\ (\text{Clog P})^2 - 0.502\ (\pm 0.372)\ \text{CMR} - 1.177\ (\pm 0.664)\ \text{I} + 8.541\ (\pm 3.133)\ \text{C log P}_o = 3.243$$
$$n = 42\ r = 0.850\ s = 0.598\ F_{5,36} = 18.69\ p < 0.01 \quad (13)$$

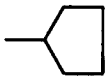
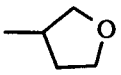
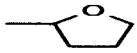
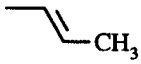
$$\text{Log } 1/IC_{50} = 1.491\ (\pm 0.604)\ \text{Clog P} - 0.230\ (\pm 0.170)\ (\text{Clog P})^2 + 1.022\ (\pm 0.491)\ \sigma_p - 1.179\ (\pm 0.539)\ \text{I} + 4.964\ (\pm 0.519)\ \text{Clog P}_o = 3.241$$
$$n = 35\ r = 0.906\ s = 0.483\ F_{5,36} = 34.20\ p < 0.0 \quad (14)$$

The enzyme inhibitory activities and the physicochemical parameters used in the regression analysis of the side chain 3-substituted analogues of the active metabolite of leflunomide are summarized in Table III.

The results of stepwise regression analysis are shown in equations 15–19. From equations 15 and 18, one can see that

**Table III.** The Enzyme Inhibitory Activities and the Physicochemical Parameters Used in the Regression Analysis of the Side Chain 3-Substituted Analogues



| Compd No. | R | Mouse DHODH IC$_{50}$[a] (nM) | Mouse DHODH log 1/IC$_{50}$(M) obsd. | Mouse DHODH log 1/IC$_{50}$(M) calcd.[b] | Rat DHODH IC$_{50}$[a] (nM) | Rat DHODH log 1/IC$_{50}$ (M) obsd. | Rat DHODH log 1/IC$_{50}$ (M) calcd.[c] | Clog P[d] | CMR[d] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | —$CH_3$ | 69 | 7.16 | 7.25 | 13 | 7.89 | 8.23 | 2.10 | 6.14 |
| 2 |  | 47 | 7.33 | 7.04 | 21 | 7.68 | 7.09 | 2.54 | 6.93 |
| 3 |  | 209 | 6.68 | 6.43 | 282 | 6.55 | 6.20 | 3.10 | 7.35 |
| 4 |  | 28200 | 4.45 | 4.93 | 31600 | 4.50 | 4.66 | 3.67 | 7.82 |
| 5 |  | 11700 | 4.93 | 4.28 | 17800 | 4.75 | 4.19 | 1.32 | 7.51 |
| 6 |  | 7940 | 5.10 | 5.76 | 44700 | 4.35 | 5.28 | 1.83 | 7.51 |
| 7 |  | 92 | 7.04 | 6.73 | 295 | 6.53 | 6.78 | 3.02 | 7.07 |
| 8 |  | 195 | 6.71 | 6.75 | 251 | 6.60 | 6.32 | 2.60 | 7.35 |
| 9 |  | 178 | 6.75 | 6.84 | 23 | 7.64 | 6.95 | 2.20 | 6.88 |
| 10 |  | 2090 | 5.68 | 6.15 | 500 | 6.30 | 6.65 | 1.75 | 6.71 |
| 11 |  | 4270 | 5.37[e] | (6.73) | 1410 | 5.85 | 6.34 | 2.73 | 7.35 |
| 12 | —$CH_2CH{=}CH_2$ | 63 | 7.20 | 6.97 | 107 | 6.97 | 6.91 | 2.67 | 7.04 |

[a] From ref. 7.
[b] Calculated from equation 17.
[c] Calculated from equation 19.
[d] Calculated values using the CQSAR program.
[e] A statistical outlier, not included in equation 17.

CMR makes the most important contribution to the inhibitory activity against DHODH in both mouse and rat enzymes, followed by Clog P and (Clog P)$^2$. From equation 16, compound **11** (see Table III) with a methyl vinyl (propenyl) function as R behaved as a statistical outlier. This may be due to the fact that compound **11** having almost the same Clog P and CMR values but quite different IC$_{50}$ values as compared with those of compound **12** with an allyl function as R. It is known that vinyl and allyl groups have quite different electronic properties as reflected by the different chemical stabilities of vinylchloride vs allylchloride. This difference is not represented by Clog P and CMR. Because of the limited number of data points with different electronic properties, addition of electronic parameter can not be justified. After deleting this outlier, equation 17 was obtained with an improved $r$ and a decreased $s$. Equations 17 and 19 are the best ones for the side chain 3-substituted analogues in mouse and rat enzymes, respectively. These results suggest that increasing the size of

the substituent reduces the inhibitory activity against DHODH in both species. The inhibitory activities against DHODH in both species are parabolically dependent on Clog P. To achieve maximum inhibitory activities in mouse DHODH and rat DHODH, an optimal Clog $P_0$ value of 2.610 and 2.733 is required, respectively, which is close to that of compound **2**, the best compound with a cyclopropyl substitutent. Here, a cyclopropyl group is confirmed to be the best 3-substituent by the QSAR analysis. An optimal Clog P value plays an important role for transport of compounds to the binding site, while the size of 3-substituent determines the interaction between the 3-substituent and its restricted binding site on the enzyme DHODH. The squared correlation matrix ($r^2 = 0.107$ between Clog P and CMR) for the parameters used in this data set demonstrates that Clog P and CMR are independent of each other because of the presence of both polar and nonpolar groups. In this data set, it should be mentioned that the degree of freedom was limited as compared with the parameters used in the regression analysis because of the limited number of data points.

*Mouse DHODH*

$$\text{Log } 1/IC_{50} = -1.514(\pm 1.224)\text{CMR} + 17.002\ (\pm 8.751)$$
$$n = 12\ r = 0.657\ s = 0.810\ F_{1,10} = 7.45\ p < 0.05 \quad (15)$$

$$\text{Log } 1/IC_{50} = -1.054(\pm 1.261)\text{CMR} + 5.800\ (\pm 5.234)$$
$$\text{C log P} - 1.093(\pm 1.086)(\text{Clog P})^2 + 6.504(\pm 13.015)$$
$$n = 12\ r = 0.856\ s = 0.620\ F_{3,8} = 7.29\ p < 0.05 \quad (16)$$

$$\text{Log } 1/IC_{50} = -0.726(\pm 1.048)\text{CMR} + 7.439\ (\pm 4.432)$$
$$\text{C log P} - 1.425(\pm 0.917)(\text{Clog P})^2 + 2.377(\pm 11.037)$$
$$\text{Clog } P_o = 2.610$$
$$n = 11\ r = 0.922\ s = 0.480\ F_{3,7} = 13.52\ p < 0.01 \quad (17)$$

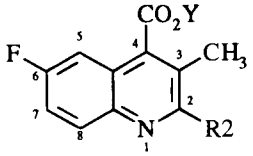
*Rat DHODH*

$$\text{Log } 1/IC_{50} = -2.201(\pm 1.027)\text{CMR} + 22.010\ (\pm 8.303)$$
$$n = 12\ r = 0.799\ s = 0.773\ F_{1,10} = 17.63\ p < 0.01 \quad (18)$$

$$\text{Log } 1/IC_{50} = -1.882(\pm 1.018)\text{CMR} + 5.067\ (\pm 4.226)$$
$$\text{Clog P} - 0.927(\pm 0.876)(\text{Clog P})^2 + 13.242(\pm 12.273)$$
$$\text{Clog } P_o = 2.733$$
$$n = 12\ r = 0.912\ s = 0.589\ F_{3,8} = 13.20\ p < 0.01 \quad (19)$$

In summary, the QSAR analysis of analogues of the active metabolite of leflunomide has identified the structural requirements for optimum activity as inhibitors of DHODH: (I) at para-position (substituent X), an electron-withdrawing group with a

**Table IV.** The Enzyme Inhibitory Activities Against Murine Leukemia L1210 Mitochondria and the Physicochemical Parameters Used in the Regression Analysis of $R_2$ Substituted 6-fluoro-3-methyl-4-quinoline Carboxylic Acids/Salts




**Y = H or Na**

| Compd No. | $R_2$ | Ki[a] (nM) | log 1/Ki(M) obsd. | log 1/Ki(M) calcd.[b] | Clog P[c] | CMR[c] |
|---|---|---|---|---|---|---|
| 1 | 4-(3-F-$C_6H_4$)$C_6H_4$ | 52.2 | 7.28 | 7.40 | 6.76 | 10.34 |
| 2 | 4-(4-F-$C_6H_4$)$C_6H_4$ | 58.7 | 7.23 | 7.40 | 6.76 | 10.34 |
| 3 | 4-(4-$CH_3$-$C_6H_4$)$C_6H_4$ | 2.1 | 8.68 | 7.51 | 7.12 | 10.78 |
| 4 | 4-(4-OH-$C_6H_4$)$C_6H_4$ | 39.3 | 7.41 | 6.77 | 5.95 | 10.47 |
| 5 | 4-($C_6H_5$)$C_6H_4$ | 23.5 | 7.63 | 7.30 | 6.62 | 10.32 |
| 6 | 4-(4-Br-$C_6H_4$)$C_6H_4$ | 112.0 | 6.95 | 7.61 | 7.48 | 11.10 |
| 7 | 4-(*c*-$C_6H_{11}$)$C_6H_4$ | 27.5 | 7.56 | 7.82 | 7.35 | 10.41 |
| 8 | 4-(*c*-$C_6H_9$)$C_6H_4$ | 892.0 | 6.05[d] | (7.50) | 6.87 | 10.39 |
| 9 | 4-(piperidine)$C_6H_4$ | 3690.0 | 5.43 | 6.02 | 4.88 | 10.32 |
| 10 | 4-(*n*-$C_6H_{13}$)$C_6H_4$ | 46.3 | 7.33 | 8.15 | 7.88 | 10.59 |
| 11 | 4-(*n*-$C_4H_9O$)$C_6H_4$ | 121.0 | 6.92 | 7.14 | 6.33 | 9.82 |
| 12 | 4-(*n*-$CH_3O$)$C_6H_4$ | 3710.0 | 5.43 | 5.41 | 4.74 | 8.43 |
| 13 | 4-($C_2H_5$)$C_6H_4$ | 93.5 | 7.03 | 6.37 | 5.76 | 8.74 |
| 14 | 4-(Br)$C_6H_4$ | 3030.0 | 5.52 | 6.16 | 5.60 | 8.59 |
| 15 | $C_6H_5$ | 40300.0 | 4.40 | 4.83 | 4.73 | 7.81 |
| 16 | 4-(*t*-butyl)$C_6H_4$ | 7.8 | 8.11 | 7.29 | 6.56 | 9.66 |
| 17 | 4-(($CH_3$)$_2$CHS)$C_6H_4$ | 36.8 | 7.43 | 7.05 | 6.22 | 10.01 |

Table IV. *(continued)*

| Compd No. | $R_2$ | Ki[a] (nM) | log 1/Ki(M) obsd. | log 1/Ki(M) calcd.[b] | Clog P[c] | CMR[c] |
|---|---|---|---|---|---|---|
| 18 | 4-$((CH_3)_2CHSO_2)C_6H_4$ | 6490.0 | 5.19 | 5.41 | 4.02 | 10.07 |
| 19 | 4-$(C_6H_5O)C_6H_4$ | 25.7 | 7.59 | 7.49 | 6.92 | 10.47 |
| 20 | 4-$(C_6H_5CH_2)C_6H_4$ | 41.7 | 7.38 | 7.27 | 6.80 | 10.78 |
| 21 | 4-$(C_6H_5S)C_6H_4$ | 36.1 | 7.44 | 7.35 | 7.16 | 11.13 |
| 22 | 4-$(C_6H_5SO_2)C_6H_4$ | 1900.0 | 5.72 | 5.79 | 5.11 | 11.19 |
| 23 | 4-$(C_6H_5SO)C_6H_4$ | 4300.0 | 5.37 | 5.56 | 4.78 | 11.16 |
| 24 | 4-$(C_6H_5CH_2S)C_6H_4$ | 8.9 | 8.05 | 6.84 | 6.95 | 11.59 |
| 25 | 4-$(C_6H_5CH_2CH_2)C_6H_4$ | 105.0 | 6.98 | 7.28 | 7.18 | 11.25 |
| 26 | 5,6,7,8-$H_4$-naphthalene-2-yl | 21.4 | 7.67 | 7.35 | 8.19 | 12.00 |
| 27 | 4-$(C_6H_5)C_6H_4$-CH=CH- | 20500.0 | 4.69[d] | (7.11) | 7.02 | 11.37 |
| 28 | 3-$(C_6H_5)$-4-$(CH_3O)C_6H_3$ | 80.6 | 7.09 | 7.00 | 6.54 | 10.94 |
| 29 | Furfur-2-yl | 261000.0 | 3.58 | 3.37 | 4.12 | 7.02 |
| 30 | 4-(2-F-$C_6H_4)C_6H_4$ | 25.0 | 7.60 | 7.40 | 6.76 | 10.34 |
| 31 | 4-(4-$C_2H_5$-$C_6H_5)C_6H_4$ | 18.3 | 7.74 | 7.63 | 7.65 | 11.25 |
| 32 | 4-(2,4-$F_2$-$C_6H_3)C_6H_4$ | 52.0 | 7.28 | 7.51 | 6.91 | 10.35 |
| 33 | 4-(3,4-$(CH_3)_2$-$C_6H_3)C_6H_4$ | 45.3 | 7.34 | 7.57 | 7.57 | 11.25 |
| 34 | 4-(3-Cl-4-$CH_3$-$C_6H_3)C_6H_4$ | 49.7 | 7.30 | 7.75 | 7.83 | 11.28 |
| 35 | 4-(3,4-$Cl_2$-$C_6H_3)C_6H_4$ | 3530.0 | 5.45[d] | (7.84) | 7.93 | 11.30 |
| 36 | 4-(2-thenyl)$C_6H_4$ | 50.3 | 7.30 | 7.48 | 6.97 | 10.59 |
| 37 | Naphthalene-2-yl | 62.8 | 7.20 | 7.05 | 7.79 | 12.01 |
| 38 | Naphthalene-1-yl | 437.0 | 6.36 | 7.05 | 7.79 | 12.01 |
| 39 | 2-$(CH_3)$-4-$(CH_6H_5)C_6H_3$ | 73.2 | 7.14 | 7.29 | 6.82 | 10.78 |

[a] From ref. 6. The apparent inhibition constant (Ki) was determined by a Dixon plot using the plot of 1/rate of orotate formation at a fixed concentration of dihydroorotate (10 μM) vs. test analog concentration, as described in ref. 6.
[b] Calculated from equation 22.
[c] Calculated values using the CQSAR program.
[d] Statistical outliers, not included in equation 22.

large π and a small MR value is preferred; (II) compounds with an ortho-substituent (substituent Z) or an electron-withdrawing substituent at meta-position (substituent Y) drastically reduce the inhibitory activity; (III) a cyclopropyl group is confirmed to be an ideal substituent at 3 position.

### Analogues of Quinoline Carboxylic Acids

The enzymatic inhibitory activities of sixty-nine quinoline carboxylic acids and their corresponding sodium salts were determined by Chen *et al.* (6). In all analogues tested, the free carboxylic acid and its corresponding sodium salt inhibited DHODH almost equally. During the calculation of Clog P, the unionized carboxy group (-COOH) was used for both the free acid and its sodium salt, since under physicological condition an equilibrium exists between the ionized form and the unionized from depending on the pKa-pH values (11). Since -COOH group is separated from the polar substituents of $R_2$ by two benzene rings, the difference in the pKa values will be very small. Thus the very slight different degrees of ionization can be neglected during the regression analysis, whether the ionized or the unionized form is used will not affect the correlation, but only change the intercept term (11).

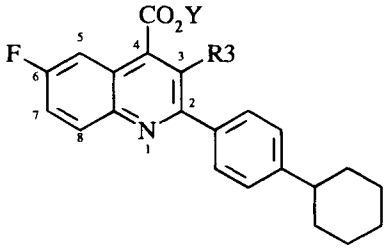
The biological activities and physicochemical parameters used in the QSAR analysis of $R_2$ substituted 6-fluoro-3-methyl-4-quinoline carboxylic acids/salts are listed in Table IV.

From equations 20 to 22, it has been found that Clog P and CMR make the major contribution to the inhibitory activity. The optimal CMR was found to be 9.884. The biphenyl group can be replaced with an appropriate bulky hydrophobic group with almost the same Clog P and CMR values, e.g. cyclohexylphenyl or 4-*t*-butylphenyl or a fused ring as described by Chen *et al.* (6). The substitution on the second phenyl ring generally does not affect the binding affinity toward DHODH when the Clog P and CMR are constants. The same can be said for the insertion of one or two atoms between the biphenyl rings. These results suggest that the interaction between $R_2$ substituent and its corresponding binding site on DHODH are mainly hydrophobic and steric interactions. The squared correlation matrix ($r^2$) between Clog P and CMR was found to be 0.490. This is due to the most $R_2$ substituents presenting as nonpolar groups.

*Mouse DHODH*

$$\text{Log } 1/\text{Ki} = 0.673(\pm 0.260)\,\text{Clog P} + 2.340\ (\pm 1.731)$$
$$n = 39\ r = 0.652\ s = 0.872\ F_{1,37} = 27.41\ p < 0.01 \qquad (20)$$

**Table V.** The Enzyme Inhibitory Activities and the Physicochemical Parameter Used in the Regression Analysis of $R_3$ Substituted 6-fluoro-3-methyl-4-quinoline Carboxylic Acids/Salts



Y = H or Na

| Compd No. | $R_3$ | Ki[a] (nM) | log 1/Ki (M) obsd. | log 1/Ki (M) calcd.[b] | $MR_{R3}$[c] |
|---|---|---|---|---|---|
| 1 | H | 122.0 | 6.91 | 6.94 | 0.10 |
| 2 | $CH_3$[d] | 27.5 | 7.56 | 7.54 | 0.57 |
| 3 | $CH_3$[e] | 25.5 | 7.59 | 7.54 | 0.57 |
| 4 | $C_2H_5$ | 111.0 | 6.96 | 7.03 | 1.03 |
| 5 | $C_3H_7$ | 2870.0 | 5.54 | 5.52 | 1.50 |

[a] From ref. 6.
[b] Calculated from equation 23.
[c] Obtained from the CQSAR program.
[d] Y = H.
[e] Y = Na.

$$\text{Log } 1/\text{Ki} = 0.664\ (\pm 0.314)\ \text{Clog P} + 5.441\ (\pm 2.852)\ \text{CMR} - 0.277(\pm 0.145)(\text{CMR})^2 - 23.861(\pm 13.836)$$
$$\text{CMR} = 0.423$$
$$n = 33\ r = 0.886\ s = 0.486\ F_{4,28} = 25.35\ p < 0.01 \quad (21)$$

$$\text{Log } 1/\text{Ki} = 0.741(\pm 0.213)\ \text{Clog P} + 5.145\ (\pm 1.928)\ \text{CMR} - 0.260(\pm 0.098)(\text{CMR})^2 - 22.979(\pm 9.337)$$
$$\text{CMR}_0 = 9.884$$
$$n = 36\ r = 0.899\ s = 0.502\ F_{3,32} = 44.94\ p < 0.01 \quad (22)$$

From equation 23, it is confirmed that the compound with a methyl subtitution at 3 position is the best inhibitor. The inhibitory activity is parabolically dependent on the MR although the data points are limited ($n = 5$). When the methyl group is replaced by a hydrogen or an ethyl/propyl group, the inhibitory activity is reduced (see Table V).

The biological activities and physicochemical parameters used in the QSAR analysis of $R_2$ and $R_6$ substituted 3-methyl-4-quinoline carboxylic acids/salts are presented in Table VI.

From equations 24–27, it appears that $\pi_{R6}$ and $\sigma_{p\,R6}$ make a positive contribution, while $MR_{R6}$ makes a negative contribution to the inhibitory activity. $R_2$ group does not affect the relative inhibitory activity because of its constant parameter values, e.g. $\pi$ and MR. Electron-withdrawing groups (F, Cl and $CF_3$) with larger $\pi$ and $\sigma_p$ values and a smaller MR value at 6-position give good inhibitory activities. However, other electron-withdrawing polar hydrophilic groups ($NO_2$, $SO_2CH_3$, COONa and $COOCH_3$) result in less active or inactive analogues because of a higher MR value and a lower or negative $\pi$ value. Electron-donating groups ($-CH_3$, $-C_2H_5$, $-NH_2$, -OH and $-OCH_3$) with smaller or negative $\pi$ and $\sigma_p$ values also reduce the enzyme inhibitory activity. The squared correlation matrix for the parameters used in the regression analysis indicates that $\pi_{R6}$, $\sigma_{pR6}$ and $MR_{R6}$ are independent of each other ($r^2 = 0.007$ between $\pi_{R6}$ and $\sigma_{pR6}$, $r^2 = 0.020$ between $\pi_{R6}$ and $MR_{R6}$, and $r^2 = 0.119$ between $\sigma_{pR6}$ and $MR_{pR6}$ respectively). Equation 27 was found to be the best equation with an $r$ of 0.960, and to be statistically significant at 99% significance level. Furthermore, by using this equation, 92.2% ($r^2 = 0.922$) of the variance in the data can be accounted for.

*Mouse DHODH*

$$\text{Log } 1/\text{Ki} = 2.900(\pm 1.430)\,MR_{R3} - 2.459\ (\pm 0.844) - (MR_{R3})^2 + 6.674\ (\pm 0.498)$$
$$n = 5\ r = 0.997\ s = 0.092\ F_{2,2} = 161.59\ p < 0.01$$
$$MR_{R3\,o} = 0.590 \quad (23)$$

$$\text{Log } 1/\text{Ki} = 0.743\ (\pm 0.335)\ \pi_{R6} + 6.205\ (\pm 0.449)$$
$$n = 16\ r = 0.786\ s = 0.832\ F_{1,14} = 22.68\ p < 0.01 \quad (24)$$

$$\text{Log } 1/\text{Ki} = 0.795(\pm 0.277)\,\pi_{R6} - 1.161\ (\pm 0.882)MR_{R6} + 7.093(\pm 0.769)$$
$$n = 16\ r = 0.874\ s = 0.678\ F_{2,13} = 21.12\ p < 0.01 \quad (25)$$

$$\text{Log } 1/\text{Ki} = 0.783(\pm 0.231)\,\pi_{R6} - 1.483\ (\pm 0.779)MR_{R6} + 1.048(\pm 0.854)\sigma_{p\,R_6} + 7.233(\pm 0.649)$$
$$n = 16\ r = 0.923\ s = 0.558\ F_{3,12} = 23.12\ p < 0.01 \quad (26)$$

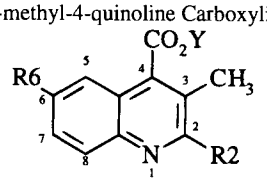
$$\text{Log } 1/\text{Ki} = 0.776\ (\pm 0.168)\ \pi_{R6} - 1.234\ (\pm 0.587)MR_{R6} + 1.192(\pm 0.627)\sigma_{pR6} + 7.126(\pm 0.476)$$
$$n = 15\ r = 0.960\ s = 0.401\ F_{3,11} = 43.33\ p < 0.01 \quad (27)$$

By using the QSAR equations here, we have quantitatively advanced the conclusion drawn by Chen *et al.* (6). The four principal regions are: (I) the 2-position where bulky hydrophobic substituent with an optimal MR of 9.884 is necessary; (II) the 3-position where a methyl group is the best substituent; (III) the 6-position where a electron-withdrawing group with a large $\pi$ value and a small MR value is an ideal substituent; (IV) the 4-position which has a strict requirement for the carboxylic acid or its corresponding salt. There is an important ionic interaction between the carboxy group of the brequinar sodium analogues and a positively charged group of DHODH.

## CONCLUSIONS

From the QSAR analysis described above, two series of compounds were found to have different optimal structural requirements. Besides the different sources of enzyme used, the critical regions of the two series of compounds are also different. In addition, comparison of the Clog P and CMR values of HR 325 with those of brequinar sodium, the best compounds in these two series of analogues, one can see that significant differ-

**Table VI.** The Enzyme Inhibitory Activities and the Physicochemical Parameters Used in the Regression Analysis of $R_2$ and $R_6$ Substituted 3-methyl-4-quinoline Carboxylic Acids/Salts




Y = H or Na

| Compd No. | $R_2$ | $R_6$ | Ki[a] (nM) | log 1/Ki (M) obsd. | log 1/Ki (M) calcd.[b] | $\pi_{R6}$[c] | $\sigma_{p\,R6}$[c] | $MR_{R6}$[c] |
|---|---|---|---|---|---|---|---|---|
| 1 | 4-($C_6H_5$)$C_6H_4$ | H | 136.0 | 6.87 | 7.00 | 0.00 | 0.00 | 0.10 |
| 2 | 4-(2-F-$C_6H_5$)$C_6H_4$ | F | 25.0 | 7.60 | 7.19 | 0.14 | 0.06 | 0.09 |
| 3 | 4-(2-F-$C_6H_5$)$C_6H_4$ | Cl | 36.6 | 7.44 | 7.21 | 0.71 | 0.23 | 0.60 |
| 4 | 4-($C_6H_5$)$C_6H_4$ | Br | 77.3 | 7.11 | 6.97 | 0.86 | 0.23 | 0.89 |
| 5 | 4-(2-I-$C_6H_5$)$C_6H_4$ | I | 242.0 | 6.62 | 6.49 | 1.12 | 0.18 | 1.39 |
| 6 | 4-($C_6H_5$)$C_6H_4$ | $CF_3$ | 9.2 | 8.04 | 7.83 | 0.88 | 0.54 | 0.50 |
| 7 | 4-(*c*-$C_6H_{11}$)$C_6H_4$ | $CH_3$ | 442.0 | 6.36 | 6.66 | 0.56 | –0.17 | 0.57 |
| 8 | 4-(*c*-$C_6H_{11}$)$C_6H_4$ | $C_2H_5$ | 171.8 | 6.77 | 6.47 | 1.02 | –0.15 | 1.03 |
| 9 | 4-(*c*-$C_6H_{11}$)$C_6H_4$ | $NO_2$ | 190.3 | 6.72 | 6.93 | –0.28 | 0.78 | 0.74 |
| 10 | 4-(*c*-$C_6H_{11}$)$C_6H_4$ | $NH_2$ | 15700.0 | 4.80 | 4.72 | –1.23 | –0.66 | 0.54 |
| 11 | 4-(*c*-$C_6H_{11}$)$C_6H_4$ | OH | 6230.0 | 5.21 | 5.81 | –0.67 | –0.37 | 0.29 |
| 12 | 4-(*c*-$C_6H_{11}$)$C_6H_4$ | $OCH_3$ | 4670.0 | 5.33 | 5.82 | –0.02 | –0.27 | 0.79 |
| 13 | 4-($C_6H_5$)$C_6H_4$ | $SCH_3$ | 676.0 | 6.17 | 5.89 | 0.61 | 0.00 | 1.38 |
| 14 | 4-(*c*-$C_6H_{11}$)$C_6H_4$ | $SO_2CH_3$ | 30200.0 | 4.52 | 5.06 | –1.63 | 0.72 | 1.35 |
| 15 | 4-($C_6H_5$)$C_6H_4$ | COONa | 311000.0 | 3.51 | 3.00 | –0.32[e] | 0.45[e] | 0.69[e] |
| 16 | 4-(*c*-$C_6H_{11}$)$C_6H_4$ | $COOCH_3$ | 30400.0 | 4.52[d] | (6.06) | –0.01 | 0.45 | 1.29 |

[a] From ref. 6.
[b] Calculated from eq. 27.
[c] Obtained from the CQSAR program.
[d] A statistical outlier, not included in eq. 27.
[e] Using the values of $COO^-$, because the —COONa group at $R_6$ position will be ionized under the testing condition.

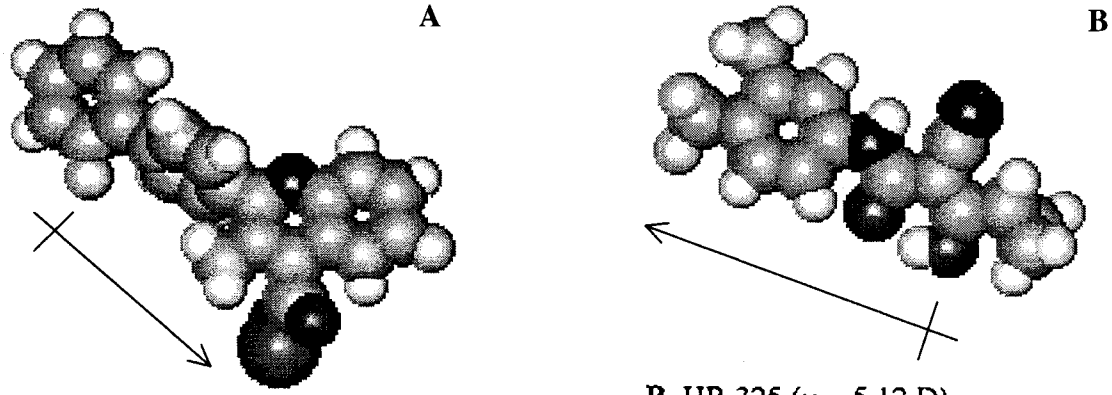



**Fig. 2.** The space-filled models of brequinar sodium (**A**) and HR 325 (**B**). Because of the rigid ring system between the quinoline and the biphenyl in **A**, there is very limited degree of free rotation or conformational change. In **B** the side chain is also rigid and coplanar due to the presence of three sp2 carbon atoms in conjugation with the rest of the molecule (see Figure 1).

ences exit between them. Furthermore, only partial overlap can been shown by superimposition of the 3-dimensional structures of HR 325 on that of brequinar sodium (7). The different space-filled models of HR 325 and brequinar sodium are shown in Figure 2. In both most active compounds they exit as rigid structures due to the aromatic ring systems in brequinar sodium and the conjugated side chain in HR325. The unionized forms of the molecules have relatively high molecular dipole moments of 3.46 D and 5.12 D, respectively. It appears that HR 325 and brequinar sodium probably bind to different sites on DHODH. This is more likely than having different kinetically determined rate-limiting steps. Studies to determine whether HR 325 and brequinar bind to the same site on DHODH or not are being pursued by Kuo and coworkers (7).

Statistically significant correlations were obtained by using a combination of 3–4 parameters. Several key structural requirements of two series of DHODH inhibitors have been identified. It is likely that two series of DHODH inhibitors bind to different binding sites on DHODH. These results provide a better understanding of the intermolecular forces involved in DHODH inhibitor-enzyme interactions, and may be useful for further modification and improvement of DHODH inhibitors.

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