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Crystal structures of *Candida albicans* dihydrofolate reductase bound to propargyl-linked antifolates reveal the flexibility of active site loop residues critical for ligand potency and selectivity

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Abstract

Candida albicans and *Candida glabrata* cause fungal bloodstream infections that are associated with significant mortality. As part of an effort to develop potent and selective antifolates that target dihydrofolate reductase (DHFR) from *Candida* species, we report three ternary crystal structures of *Candida albicans* DHFR (CaDHFR) bound to novel propargyl-linked analogs. Consistent with earlier modeling results, these structures show that hydrophobic pockets in the binding site may be exploited to increase ligand potency. The crystal structures also confirm that loop residues Thr 58- Phe 66, which flank the active site and influence ligand potency and selectivity, adopt multiple conformations. To aid the development of a dual *Candida* spp. inhibitor, three new crystal structures of *C. glabrata* DHFR (CgDHFR) bound to similar ligands as those bound in the ternary structures of CaDHFR are also reported here. Loop residues 58-66 in CgDHFR and human DHFR are 1 Å and 3 Å closer to the folate binding site, respectively, than loop residues in CaDHFR, suggesting that a properly size ligand could be a potent and selective dual inhibitor of CaDHFR and CgDHFR.

Keywords

Candida albicans; *Candida glabrata*; X-ray crystallography; structure-based drug design; DHFR; antifolate

INTRODUCTION

Opportunistic fungal infections have increased significantly over the last few decades with candidemia bloodstream infections (BSI) being the most prevalent (1-3). In fact, the incidence of hospitalization due to Candidemia in the United States has doubled in just five years (4). *Candida albicans* continues to account for the majority of Candidemia infections (5). However, other species namely *Candida glabrata*, have also emerged as important fungal pathogens (5-7). Complicating this problem, *C. glabrata* has been shown to express efflux pumps, inducing resistance to the clinically used azole antifungal agents (8,9). The

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SUPPORTING INFORMATION

Supporting information, including two figures illustrating the electron density for the active site of the CaDHFR crystal structures and a superposition of the active sites of the CaDHFR and huDHFR structures, is available through the online edition.

emergence of resistant strains, coupled with mortality rates at nearly 50 % (10), suggest a need for new therapeutics.

Dihydrofolate reductase (DHFR) is a well established drug target that is essential for DNA and protein synthesis (11-14). In previous work, we developed models of *Candida albicans* dihydrofolate reductase (CaDHFR) and docked a series of propargyl-linked antifolates. The docking models illustrate structure-activity relationships (SAR) and reveal opportunities for designing additional propargyl-linked analogs that may exhibit increased inhibition of CaDHFR. In brief, modifications at the following positions are predicted to enhance potency: increasing the length of the alkyl chain at the C6 position of the diaminopyrimidine ring (see Table 1 for a description of positions) to form hydrophobic contacts with Thr 58, Ile 62, Leu 69 and Ile 112; increasing hydrophobicity at the propargylic position to enhance van der Waals contacts with Met 25, Ile 33 and Phe 36; and increasing hydrophobic substitutions on the phenyl ring to form contacts with Phe 36, Ile 62, Phe 66 and Leu 69 (15).

In this investigation we report three new ternary CaDHFR crystal structures bound to the cofactor, NADPH and propargyl-linked antifolates. These new structures validate the previous CaDHFR docking studies and SAR. In addition, comparisons of these three CaDHFR structures and three new *Candida glabrata* dihydrofolate reductase (CgDHFR) structures bound to the same ligands, also reported here, advance the future design of selective dual inhibitors. Comparisons of these CaDHFR structures bound to the propargyl-linked antifolates and previously determined structures of CaDHFR bound to 5-phenylthioquinazoline inhibitors (16) reveal the flexibility of loop residues Thr 58-Phe 66. As the interactions of loop residues Thr 58-Phe 66 with the ligand play a key role in both the selectivity and potency of the propargyl-linked ligands, the crystal structures are valuable tools for continued development of the antifolates.

METHODS AND MATERIALS

Cloning, Expression, and Purification

Genomic DNA from *C. albicans* was obtained from the American Type Culture Collection (ATCC). The CaDHFR gene was amplified by PCR and inserted into the pET-41 vector (EMD Biosciences). Resultant plasmids were used to transform *E. coli* BL21(DE3) cells. One liter cultures were grown to mid-log phase and expression was induced with 1 mM β -D-thiogalactoside. Cells were harvested after six hours by centrifugation. For protein purification, cells were lysed with Bug Buster (Novogen) and DNase I (NEB) and centrifuged. The supernatant was subjected to a 50 % ammonium sulfate precipitation overnight to remove other proteins and *E. coli* DHFR. After centrifugation the resultant supernatant was further purified using a 10 mL resin bound methotrexate column (Sigma). The column was first washed with 50 mM sodium phosphate, 1 mM dithiothreitol, 0.1 mM EDTA, 0.1 mM phenylmethanesulfonyl fluoride at pH 7.0 followed by conditioning with 1 M potassium chloride, 50 mM sodium phosphate, 1 mM dithiothreitol, 0.1 mM EDTA, 0.1 mM phenylmethanesulfonyl fluoride at pH 7.0 and elution with 3 mM dihydrofolic acid, 20 mM MES potassium salt, 20 % glycerol, 1mM dithiothreitol and 0.1 mM EDTA at pH 8.0. CaDHFR was collected and buffer was extensively exchanged ($\sim 10^7$ -fold) to 20 mM MES potassium salt, 20 % glycerol, 1mM dithiothreitol and 0.1 mM EDTA at pH 8.0 to remove residual dihydrofolic acid. Resultant protein was flash frozen and stored at -80 °C until use. Cloning, expression and protein purification of CgDHFR was performed as previously reported (17,18).

Crystallization

The hanging drop diffusion method was used to grow CaDHFR crystals. Purified CaDHFR (6-7 mg/mL) was incubated for 2 hours at 4 °C with 1 mM NADPH and 1 mM of inhibitor. The complex was concentrated to 17-20 mg/mL, mixed in a 1:1 ratio with reservoir solution containing 35-38 % PEG 3350 and 0.05 M MES potassium salt at pH 6.5. The resultant solution was mixed with the additive, glycine, to a final concentration of 0.11 M and incubated at 4 °C. Crystals were flash frozen in liquid nitrogen using 30 % glycerol as a cryoprotectant. Data were collected at Brookhaven National Laboratory using beamlines X25 and processed with HKL2000 (19). CgDHFR crystals were obtained as previously reported (17,18). High resolution data were included after evaluating data completeness, $I/\sigma(I)$ and R_{sym} . While the R_{sym} value for the data in the highest resolution bin for the Cg/NADPH/2 (PDB ID: 3QLY) complex is 41.8 % (Table 2), the data are intense ($I/\sigma(I) = 4.3$) and are therefore included. For the complex Ca/NADPH/2 (PDB ID: 3QLS), data in the shell diffracting to 1.73 Å were included because the data were redundant (3.8-fold), intense ($I/\sigma(I)$ value of 5.5) and possessed an R_{sym} value of 25.8 % (Table 2).

Structure Determination

Molecular replacement was used to determine all CaDHFR and CgDHFR structures. Initial phase information and appropriate starting models (CaDHFR or CgDHFR) were obtained using Phaser (20). Electron density was viewed using COOT (21) and refinement was carried out using Refmac 5 (22) as implemented by CCP4. Procheck was used to assess molecular geometry (23). CaDHFR in complex with compound **4** was found to be a pseudo-merohedral twin according to Wilson plots. As such, it was refined using the twin refinement feature in Refmac5. Met 25 was observed in two conformations in structures CaDHFR/NADPH/1 and CaDHFR/NADPH/2; the occupancy of each rotamer was refined to 50 %.

RESULTS

We have been developing a series of propargyl-linked antifolates as part of an antifungal drug discovery program. The propargyl-linked antifolates inhibit DHFR from *Candida albicans* (CaDHFR) and *Candida glabrata* (CgDHFR) with 50 % inhibition concentration (IC_{50}) values between 17 nM and 11.9 μM and 0.55 nM and 2.7 μM, respectively (15,17,18). In addition, they exhibit levels of selectivity over the human DHFR enzyme between 0.1- and 2300-fold (15,17). Data for five examples of these inhibitors are summarized in Table 1.

Candida albicans DHFR structures

In order to elucidate and experimentally determine the binding mode of the propargyl-linked inhibitors with CaDHFR, we determined the structures of three new complexes of CaDHFR bound to NADPH and compounds **1**, **2** and **4** by X-ray crystallography (PDB IDs: 3QLR, 3QLS and 3QLW). The ligands for crystal complex formation were chosen to explore differences at the propargylic position, the C6 position of the diaminopyrimidine ring and the substitution pattern of the phenyl ring. The CaDHFR crystals show diffraction amplitudes to 2.5 Å (3QLW), 1.73 Å (3QLS) or 2.15 Å (3QLR) (see Table 2). The structures were determined by molecular replacement using a previously reported structure of CaDHFR (PDB ID: 1AOE (24)). Electron density for the protein and ligands was well-defined (see Supporting Information Figure 1), allowing the refinement of ternary complex models.

In all of these CaDHFR structures, the protein adopts the conserved DHFR fold with an eight-stranded beta sheet and four flanking alpha helices. The ligands bind in the active site

using conserved hydrogen bonds between Glu 32, Tyr 118, Ile 9 and Ile 112 and the 2,4-diaminopyrimidine ring and van der Waals interactions between Ile 9 and Phe 36 and the pyrimidine ring. These overall binding modes observed in the crystal structures agree with those predicted in the previous docking models. Key hydrogen bonds between the pyrimidine moiety of the ligands and residues Ile 9, Glu 32, Ile 112 and Tyr 118 that are observed in docking models are also observed in crystallographic models. Additionally, important van der Waals interactions between Phe 36 and the pyrimidine ring are present in both docking and crystallographic models.

Structures of CaDHFR with compounds **1** and **2**, differing only at the propargylic position, show that the propargyl linker of both ligands forms van der Waals contacts with Thr 58, Ile 62 and Ile 112 (Figure 1A and B). The structures reveal electron density for the *R*-enantiomer of compound **1**, despite the fact that a racemic mixture was included in the crystallization mixture. In the *R* configuration, the ethyl group forms van der Waals interactions primarily with Ile 112. The *gem*-dimethyl substituent of compound **2** makes additional van der Waals interactions with the nicotinamide ring of the cofactor NADPH and Met 25 (see Figure 1B).

Enzyme inhibition results show that compound **5**, with an *n*-propyl substitution at the C6 position of the diaminopyrimidine, is slightly (2-fold) more potent than the ethyl-substituted compound **4**. The structure of CaDHFR bound to compound **4** (see Figure 1C) reveals that the pocket comprising Met 25, Ile 33 and Phe 36 surrounding the C6 position can accommodate the larger *n*-propyl substitution. These crystallographic results confirm the docking models predicting that compound **5** could form stronger van der Waals interactions with these residues.

The CaDHFR structure bound to compound **4** shows that the 2,5-dimethoxyphenyl ring is situated in a large hydrophobic pocket forming van der Waals interactions with residues Met 25, Ile 33, Phe 36, Ser 61, Ile 62 and Leu 69, all of which are within 4.5 Å of the ring (see Figure 1C). Substituting a 3,4,5-trimethoxyphenyl ring (compounds **1** and **2**) increases the van der Waals contacts, explaining the substantial increase in potency from 100 nM (compound **4**) to 18 nM (compound **3**). Relative to the dimethoxyphenyl ring, the 4'-methoxy of the trimethoxyphenyl ring forms additional contacts with Pro 63. Also, the substitution at the 4' position places the methoxy group at the optimal distance to maximize the van der Waals interactions with Leu 69, strengthening this interaction relative to the unsubstituted position (see Figure 1A and 1B).

Candida glabrata DHFR Structures

To aid the future design of dual *Candida* spp. inhibitors, three of these compounds (**1**, **2** and **5**; see Table 1) were co-crystallized with *Candida glabrata* DHFR (CgDHFR; see Table 2). The interactions of the 2,4-diaminopyrimidine ring in these structures with compounds **1**, **2** and **5** resemble the interactions observed in previously reported structures of CgDHFR bound to propargyl-linked antifolates (17,18). In CgDHFR, the ethyl and *gem*-dimethyl propargyl substitutions of compounds **1** and **2** form van der Waals interactions with hydrophobic residues Thr 58, Ile 62, and Ile 121 (see Figure 2A). The 2-fold increase in affinity for compound **2** can potentially be attributed to additional hydrophobic contacts with NADPH.

Similar to structures of CaDHFR:NADPH:**4**, the 2,5-dimethoxyphenyl ring of CgDHFR:NADPH:**5** fits in a hydrophobic pocket and forms contacts with Leu 25, Met 33, Phe 36, Thr 58, Ser 61, Ile 62, Pro 63 and NADPH (see Figure 2B). Comparing the 2,5-dimethoxyphenyl substitution (compound **5**) with the 3,4,5-trimethoxyphenyl substitution (compounds **1** and **2**) shows that the trimethoxyphenyl substitution reduces interactions with

Leu 25, Thr 58, Ser 61, and NADPH owing to the absence of a methoxy group at the 2' position. The equivalent potencies of the trimethoxyphenyl compounds **1** and **2** (27 and 11 nM respectively) and the dimethoxyphenyl compound **5** (15 nM) is explained by the additional contacts formed by the propargylic substitutions on compounds **1** and **2**.

Comparison of CaDHFR and CgDHFR structures

The C α atoms of CaDHFR:NADPH:**1** and CgDHFR:NADPH:**1** superimpose with an RMSD of 2.7 Å. Both enzymes adopt the DHFR canonical fold of a central β -sheet flanked by four α -helices. However, the CgDHFR structure shows an elongation and addition of β -strands that extends the central β -sheet beyond what is observed in the CaDHFR structure, thus accounting for the differences reflected in the RMSD (see Figure 3A). Despite the differences observed in the central β -sheet, these enzymes have structurally similar active sites with homologous hydrophobic residues interacting with the ligand at the C6 position of the diaminopyrimidine, the propargyl linker and the phenyl pocket. Interestingly, overall, the compounds possess greater affinity for CgDHFR than CaDHFR (see Table 1). The C α atoms of residues Thr 58-Phe 66 in the loop region flanking the active site shift by as much as 1 Å creating a smaller pocket to accommodate the phenyl ring in CgDHFR than in CaDHFR (see Figure 3B). The tighter pocket in CgDHFR may maximize the van der Waals interactions, leading to the observed increase in affinity (see Figure 3C). Not surprisingly, incorporating flexibility into the modeling of residues in the loop region (CaDHFR: Thr 58-Phe 66) was essential in producing docking results that correlated well with enzyme affinity (15).

Comparison to human DHFR

Since DHFR is a ubiquitous enzyme, it is essential to incorporate selectivity into any inhibitor directed at a pathogenic species. The residues of huDHFR and CaDHFR are 72 % homologous and 32 % identical (see Figure 4) and all but one, Phe 66, of the residues interacting with the propargyl-linked ligands in CaDHFR (indicated by an asterisk in Figure 4) are homologous in huDHFR. The propargyl-linked compounds currently display only modest selectivity for the fungal enzyme. However, structural analysis suggests differences that may be exploited in order to increase selectivity. Specifically, comparing the positions of C α atoms from loop residues Thr 58-Phe 66, which flank the binding site for the para-aminobenzoic acid (PABA) moiety and carboxylate tail of folic acid, in the Ca:NADPH:**2** and human DHFR (PDB ID: 1KMV) crystal structures reveals that the CaDHFR residues are located 1 to 3.7 Å further from the active site than the corresponding residues in human DHFR (see Supporting Information Figure 2). Thus, the crystal structure of CaDHFR confirms that the human DHFR folate binding site is more restricted than that of CaDHFR, potentially opening an avenue for developing increased selectivity (15).

Comparison to previously published CaDHFR structures

Comparing nine crystallographic models of CaDHFR previously published by investigators at GlaxoSmithKline (GSK), eight of which are ternary ligand-bound structures and one of which is in the binary form with no ligand, aids in elucidating the effects of different inhibitors on enzyme conformation (16,24). All of the ligand-bound GSK structures align well with the binary structure (RMSD < 0.8 Å). Likewise, the CaDHFR structures bound to the trimethoxyphenyl propargyl-linked compounds (compounds **1** and **2**) align well to the binary structure (PDB ID: 1AI9) with an RMSD < 1.2 Å. However, as shown in Figure 5A, when the binary structure is compared with CaDHFR bound to compound **4**, a dimethoxyphenyl substituted compound, a shift of up to 3 Å was observed for the C α atoms of loop residues Thr 58-Phe 66. Again it is evident that loop residues Thr 58-Phe 66 exhibit several conformations, suggesting that properly modeling the flexibility of these residues is critical to successful ligand design.

Within the active site of PDB IDs 1IA2, 1IA3, 1IA4, 1M79 and 1IA1, molecules from the buffer (4-morpholineethanesulfonic acid [MES]) or phosphate ions are bound and form hydrophilic interactions with Arg 72. MES forms additional hydrophobic interactions with the co-crystallized ligand (see Figure 5B). Interestingly, superpositions of these GSK structures with CaDHFR bound to compound **2** show that the substituted phenyl extends into the space occupied by the MES molecule. In the structures of CaDHFR bound to the propargyl-linked inhibitors, one or more water molecules coordinate with Arg72, similar to the hydrophilic tail of the MES molecule (see Figure 5B). In two of the nine GSK structures (PDB ID: 1IA4 and 1IA3) the nicotinamide portion of the NADPH structure is missing and the ligands are found in the NADPH binding site (see Figure 5C). The structures of CaDHFR bound to the propargyl-linked inhibitors did not show a displacement of NADPH. In fact, the conformation of NADPH in the CaDHFR structures here superimposes with the NADPH conformation observed in the other seven GSK structures (see Figure 5D).

DISCUSSION

Blood borne Candidemia infections are increasing in prevalence and can be lethal. Recent shifts in epidemiology including increasingly resistant strains are cause for concern. Here we report three new CaDHFR crystal structures bound to members of a new class of non-classical antifolates. The presence of the same key hydrogen bonds (Ile 9, Glu 32, Ile 112 and Tyr 113) and van der Waals interactions (Phe 36) with the pyrimidine in these crystal structures validates previous CaDHFR docking results. Like CaDHFR docking models, crystallographic structures of CaDHFR show that enzyme potency correlates with increased hydrophobicity at the propargyl linker and with increased alkyl chain length at the C6 position of the diaminopyrimidine.

The flexibility of loop residues Thr 58-Phe 66 in CaDHFR is evident from the crystal structures. Comparisons of these new CaDHFR structures with CgDHFR bound to the same ligands or to previously published CaDHFR structures (16,24) show that CaDHFR loop residues Thr 58-Phe 66 are flexible and are significantly displaced (up to 3 Å) away from the folate binding site. These experimental results explain why initial modeling and docking studies for CaDHFR and huDHFR that maintained a minimally flexible conformation for the loop residues correlated poorly with empirical enzyme inhibition assay results. Correlation with inhibition data only improved when loop residues Thr 58-Phe 66 were allowed to be more flexible via molecular dynamics simulations (15,25).

Structural insights are invaluable to understanding DHFR enzyme inhibition and guiding future inhibitor design. New crystal structures of CaDHFR reveal opportunities for increasing inhibitor potency. Specifically, a hydrophobic substitution at the 4' position of the trimethoxyphenyl is critical, forming key hydrophobic interactions with loop residues Pro 63 and Leu 69. At the C6 position of the diaminopyrimidine, both the ethyl and *n*-propyl substitutions lead to an increase in potency. Also, hydrophobic substitutions at the propargylic position lead to increasing potency for CaDHFR, with the *gem*-dimethyl and ethyl substitutions exhibiting similar potencies (45 and 33 nM, respectively).

Comparisons of CaDHFR with CgDHFR and huDHFR show that the development of selective, dual inhibitors may be possible, especially since CaDHFR and CgDHFR possess highly homologous residues. Overall, the ternary structures of CaDHFR and CgDHFR are similar, with the exception of an extended central β -sheet in CgDHFR, which is distal to the active site, and the displacement of loop residues Thr 58-Phe 66. In fact, comparisons with huDHFR show these loop residues (CaDHFR and CgDHFR: Thr 58-Phe 66, HuDHFR: Thr 56-Asn 64) are each displaced from the folate binding site at different distances and therefore, create active sites with different volumes. The loop residues in CaDHFR are

displaced the most from the folate binding site, while the huDHFR loop residues are the closest, suggesting that a properly sized ligand could be a potent and selective dual inhibitor for CaDHFR and CgDHFR.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Crystallographic models of CaDHFR bound to NADPH and A) compound **1**, B) compound **2** and C) compound **4**. Note that Met 25 has two conformations in the crystal structures with CaDHFR:NADPH:**1** and CaDHFR:NADPH:**2**.

Figure 2. Crystallographic models of CgDHFR in complex with A) compound **1** (purple) and compound **2** (cyan) B) compound **2** (pink) and compound **5** (green).

Figure 3.

A) Superposition of the secondary structural elements of CaDHFR shown in green (PDB ID: 3QLR) with CgDHFR shown in blue (PDB ID: 3QLX); note the extended central beta sheet observed in CgDHFR when compared to CaDHFR B) relative displacement of loop residues Thr 58-Phe 66 in CaDHFR (green) to CgDHFR (blue) and C) surface rendering of CgDHFR (blue mesh) and CaDHFR (red solid) in complex with compound **1** showing the differences in the sizes of the folate binding site.

CaDHFR	MSKPNVAII VAALKPALGI GYKGKMPWR--LRKEIRYFKDVTTRTTKPNTRNAVIMGRKTW	59
CgDHFR	MSKVPVVGII VAALLPEMGI GQGNLPWR--LAKEMKYFRVETTLTNDNSKQNVIMGRKTW	59
HuDHFR	--VGSINCI VAVSQ--NMGI GKNGLPWPLRNFIRYFQRMTTSSVEGKQNLVIMGKTTW	57
	* * * * *	
CaDHFR	ESI PQKFRFLPDRLNII LRSRY--ENEI--IDDNIIHAS-----SIESSLNLVSDVER	108
CgDHFR	ESI PQKFRFLPDRINVVRSRFS--DGLRKVDCGIYHASNLRNCLATQS SLANENKIER	117
HuDHFR	FSTI PEKNRFLGRINLVLSRELKEPP-----QGAHFLSRSLDDALKLTEQPELANKVDM	111
	* * * *	
CaDHFR	VFI IGGAEIYNELINNSLVSHLLITEIEHPSPES I-EMDTFLK-----	150
CgDHFR	IYI IGGGEIYRQSM D--LADHWLITKIMLPETTIPQMDTFLQKQLEQRFYDNSDKLVD	175
HuDHFR	VWI VGGSSVYKEAMNHPGHLKLVFTVIMQD-----FESDTFFP-----	149
	* * *	
CaDHFR	FPLESWTKQPKSELQKFVGDVLEDDIKEGDFTYNTLWTRK--	192
CgDHFR	FLPSSIQLERGLTISQEWNGELVGLVPVEKGYQYFTLVITYTK--	217
HuDHFR	---EIDLEKYKLPEYPG---VLSDVGEKKGIYKPFVEYKED	186

Figure 4. Structural alignment of CaDHFR, CgDHFR and huDHFR; residues contacting the ligand are indicated by an asterisk.

Figure 5.

Comparison of previously published CaDHFR structures with structures of CaDHFR bound to the propargyl-linked inhibitors A) alignment of CaDHFR GSK structures (cyan) with CaDHFR structures bound to propargyl-linked inhibitors (pink). The backbone of the binary structure PDB ID: 1AI9 (cyan) and CaDHFR bound to compound **4** (pink) are drawn in cartoon to highlight the displacement of loop residues Thr 58- Phe 66, B) placement of key water molecules and a molecule of MES in PDB ID: 1IA2 (pink) relative to compound **2** (pink), C) comparison of PDB ID 1IA4 (cyan) with CaDHFR bound to compound **2** (pink) shows displacement of NADPH by the ligand in PDB ID: 1IA4 and D) alignment of NADPH between the binary structure (PDB ID: 1AI9) of CaDHFR and compound **1** bound to CaDHFR.

Table 1

Inhibition of CaDHFR, CgDHFR and human DHFR

Ligand	Scaffold	Scaffold A		Scaffold B		IC ₅₀ (nM)	
		R ₁	R ₂	CaDHFR	CgDHFR	HuDHFR	
1	A	ethyl	methyl	45±9	27±9	200±11	
2	A	<i>gem</i> -dimethyl	methyl	33±14	11±2	290±17	
3	A	H	ethyl	18±1	6.4±0.9	57±2	
4	B	ethyl	-	100±7	8.2±1.3	1280±15	
5	B	<i>n</i> -propyl	-	45±9	15±3	1180±10	

Table 2

Crystallographic data and refinement statistics

PDB ID	Ca/NADPH/4	Ca/NADPH/2	Ca/NADPH/1	Cg/NADPH/5	Cg/NADPH/2	Cg/NADPH/1
Space group	3QLW <i>P2₁2₁2</i>	3QLS <i>P2₁</i>	3QLR <i>P2₁</i>	3QLZ <i>P4₁</i>	3QLY <i>P4₁</i>	3QLX <i>P4₁</i>
No. monomers in asymmetric unit	2	2	2	2	2	2
Unit cell (<i>a</i> , <i>b</i> , <i>c</i> in Å)	118.43, 118.65, 39.35	37.90, 66.48, 75.54	37.99, 66.27, 75.89	42.61, 42.61, 230.24	42.76, 42.76, 232.12	42.73, 42.73, 230.84
Resolution (Å)	83.93-2.50	49.88-1.73	37.90-2.15	28.78-1.94	28.69-2.24	30.22-2.52
Completeness % (last shell, %)	99.9 (100.0)	90.8 (47.3)	98.8 (98.0)	91.5 (94.1)	99.9 (100.0)	99.5 (100.0)
Unique reflections	18745	33649	19328	26275	18794	13059
Redundancy (last shell)	7.8 (7.7)	3.8 (2.3)	3.7 (3.7)	4.1 (4.6)	5.3 (5.1)	5.2 (5.3)
R _{sym} , (last shell)	0.092 (0.251)	0.085 (0.258)	0.067 (0.124)	0.110 (0.319)	0.108 (0.413)	0.082 (0.337)
< <i>I</i> /σ> (last shell)	27.7 (9.7)	28.9 (5.5)	37.4 (19.0)	26.5 (7.4)	21.6 (4.3)	29.2 (5.9)
R-factor/R _{free}	0.180/0.195	0.169/0.207	0.176/0.244	0.206/0.245	0.185/0.236	0.210/0.267
No. of atoms (protein, ligands, solvent)	3096, 46, 37	3192, 52, 337	3169, 52, 196	3692, 48, 191	3692, 52, 41	3692, 52, 74
Rms deviation bond lengths (Å), angles (deg)	0.006, 1.029	0.008, 0.950	0.010, 1.324	0.011, 0.970	0.014, 1.121	0.003, 0.697
Average B factor for protein (Å ²)	34.77	16.81	21.02	24.89	31.27	33.17
Average B factor for ligand (Å ²)	28.10	18.36	23.30	25.93	28.79	38.65
Average B factor for solvent molecules (Å ²)	33.91	25.17	23.51	28.95	24.12	28.71
Residues in most favored regions (%) ^a	90.8	92.4	92.4	93.4	91.2	92.9
Residues in additional allowed regions (%) ^a	9.2	7.6	7.6	6.1	8.3	6.6
Residues in generously allowed regions (%) ^a	0	0	0	0.5	0.5	0.5
Residues in disallowed regions (%) ^a	0	0	0	0	0	0

^a as determined by Ramachandran plot analysis