Journal of Computer-Aided Molecular Design, **14:** 495–506, 2000.
KLUWER/ESCOM

Selectivity analysis of 5-(arylthio)-2,4-diaminoquinazolines as inhibitors of *Candida albicans* dihydrofolate reductase by molecular dynamics simulations

Vijay M. Gokhale & Vithal M. Kulkarni*
Pharmaceutical Division, Department of Chemical Technology, University of Mumbai, Mumbai 400 019, India

Received 13 September 1999; Accepted 20 December 1999

Key words: docking analysis, fungal DHFR, selective inhibitors, selectivity index

Summary

A series of 5-(arylthio)-2,4-diaminoquinazolines are known as selective inhibitors of dihydrofolate reductase (DHFR) from *Candida albicans*. We have performed docking and molecular dynamics simulations of these inhibitors with *C. albicans* and human DHFR to understand the basis for selectivity of these agents. Study was performed on a selected set of 10 compounds with variation in structure and activity. Molecular dynamics simulations were performed at 300 K for 45 ps with equilibration for 10 ps. Trajectory data was analyzed on the basis of hydrogen bond interactions, energy of binding and conformational energy difference. The results indicate that hydrogen bonds formed between the compound and the active site residues are responsible for inhibition and higher potency. The selectivity index, i.e the ratio of I_{50} against human DHFR to I_{50} against fungal DHFR, is mainly determined by the conformation adapted by the compounds within the active site of two enzymes. Since the human DHFR active site is rigid, the compound is trapped in a higher energy conformation. This energy difference between the two conformations ΔE mainly governs the selectivity against fungal DHFR. The information generated from this analysis of potency and selectivity should be useful for further work in the area of antifungal research.

Introduction

Dihydrofolate reductase (DHFR) is a ubiquitous enzyme present in a variety of animal species, bacteria, bacteriophage, protozoa, and plants. DHFR is involved in cellular metabolisms and catalyzes the NADPH-dependent reduction of dihydrofolate (FH_2) to produce tetrahydrofolate (FH_4). Tetrahydrofolate and its derivatives serve as one carbon donor in the synthesis of purines, pyrimidines and several amino acids. Thus the inhibition of DHFR leads to a cellular deficiency of tetrahydrofolate cofactors, disrupting the biosynthesis of purines and pyrimidines and thus resulting in cell death [1,2]. DHFR has been identified as a target for the action of several classes of drugs important in the treatment of malaria, bacterial infections, parasitic infections and cancer. Examples of such drugs include trimethoprim, pyrimethamine and methotrexate. Availability of crystal structures of these inhibitors with DHFR from various sources resulted in the identification of potential binding interactions with enzyme and this information was used for the design of better analogues [3–5].

The increasing occurrence of systemic fungal infections in AIDS patients has resulted in the need for newer, better antifungal drugs and consequently, identification of new targets to overcome the problem of resistance. Recently, DHFR from *Candida albicans* has been identified as target for the development of antifungal agents [6]. The quinazoline class of inhibitors have been identified as antifungals [7]. GRID based analysis of *C. albicans* DHFR resulted in the discovery of pyrroloquinazolines as fungal DHFR inhibitors. This pyrroloquinazoline class of inhibitors exhibits high affinity for DHFR from *C. albicans* [8]. X-ray crystallographic analysis of fungal DHFR

*To whom correspondence should be addressed. E-mail: vithal@biogate.com

with these inhibitors suggests that they exhibit hydrogen bonding interactions similar to those observed for other quinazoline or aminopyrimidine inhibitors. Amine functional groups at the 2 and 4 positions along with ring nitrogen atoms form hydrogen bonds with the active site residues. Another factor important in the development of DHFR inhibitors as antifungals is the selectivity against fungal enzyme versus human enzyme.

Development of antifungal agents with DHFR as a target is complicated by the fact that the antifungal agent should exhibit minimum toxicity to the human host. This is important, since DHFR from *C. albicans* is more similar to human enzyme than the bacterial one. Certain 5-(arylthio)-2,4-diaminoquinazolines are reported as inhibitors of fungal DHFR and their selectivity is studied by calculation of the selectivity index, i.e. the ratio of I_{50} against human DHFR to I_{50} against fungal enzyme [7]. This selectivity is governed by the binding characteristics and interaction of the inhibitors with fungal as well as human enzyme.

Molecular mechanics based methods involving docking studies and molecular dynamics simulations have been used to study the binding orientations and prediction of binding affinities. Such studies have been applied in case of acetylcholinesterase inhibitors [9,10], interleukin-1β converting enzyme (ICE) inhibitors [11], aldose reductase inhibitors [12], bradykinin β2 receptor antagonists [13] and protein kinase inhibitors as staurosporine [14]. In some cases, these studies have been used to predict the binding affinities quantitatively, as in the case of Herpes simplex virus thymidine kinase inhibitors [15]. In some cases such studies have been used in conjunction with mutagenesis studies for active site identification, as in the case of phorbol esters binding to protein kinase C [16].

We have used molecular docking and dynamics simulations to study the selectivity of 5-(arylthio)-2,4-diaminoquinazolines for *C. albicans* DHFR and present here a comparative study of binding interactions of these inhibitors with fungal and human DHFR.

Methods

All molecular modeling studies were carried out using Insight II 97.0 [17] running on a Silicon Graphics Indigo2 Solid Impact R4400 workstation. All the minimizations and molecular dynamics studies were performed using the extensible systematic force field (ESFF) within Discover 3.0.

Enzyme structure

Starting enzyme structures of *C. albicans* DHFR (PDB entry 1aoe) and human DHFR (PDB entry 1ohk) were obtained from the Protein Data Bank of Brookhaven National Laboratory.

All the hydrogens were added and enzyme structures were subjected to a refinement protocol in which the constraints on the enzyme were gradually removed and minimized until the rms gradient was 0.01 kcal/mol Å. The energy minimization was carried out using the Discover 3.0 program within Insight II. These energy-minimized structures were used for docking studies.

Compounds

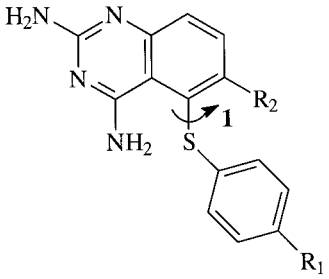
A series of *C. albicans* DHFR inhibitors belonging to the class of 5-arylthio-2,4-diaminoquinazolines were selected for the present study. Enzyme inhibitory activity in terms of I_{50} (μM) against *C. albicans* and human DHFR are reported in Table 1. All the compounds were constructed using the Builder module starting from pyrroloquinazoline from the crystal structure complex. Partial atomic charges were assigned using ESFF and minimized using Discover 3.0 [18]. Conformational search was carried out around two important rotatable bonds with an increment of 10° using the Search_Compare module. All the conformers were minimized until the rms deviation was 0.01 kcal/mol Å. Low energy conformers were clustered and conformers with unique geometry were selected for docking and MD simulations. For all the compounds, global minimum conformations were similar.

Docking and molecular dynamics simulations

The docking and MD simulations were carried out on *C. albicans* and human enzyme for each inhibitor. Active sites were defined using a radius of 12.0 Å around the inhibitor in case of both the enzymes and MD simulations were performed on active sites with the rest of the enzyme fixed. Cofactor nicotinamide adenine dinucleotide phosphate (NADPH), considered as a part of the enzyme structure, was not fixed during the simulations.

Each compound was docked into the active site using the crystal structure ligand as a template for the initial placement. The compound was moved and/or rotated to reduce the bumps. This initial model was

Table 1. Structures and activities of compounds used in the study with torsion 1 labeled



Compound	R_1	R_2	I_{50} *C.a*[a] (μM)	I_{50}H[b] (μM)	S.I.[c]
1	-$C(CH_3)_3$	-H	0.008	2.0	250
2	-$(CH_2)_5CH_3$	-H	0.04	0.55	14
3	cyclohexyl	-H	0.05	>10	>200
4	morpholinyl	-H	0.13	70	540
5	-OH	-H	0.034	0.18	5.0
6	-OCH_3	-H	0.020	3.5	175
7	-CF_3	-H	0.48	2.6	5.0
8	-$C(CH_3)_3$	-NH_2	0.25	4.3	17
9	-$C(CH_3)_3$	isobutoxy	0.003	0.030	10
10	3,4,5-(-$OCH_3)_3$	-H	0.04	4.4	110

[a] I_{50} value against *Candida albicans* DHFR.
[b] I_{50} value against human DHFR.
[c] S.I. (selectivity index), defined as: S.I. = (I_{50} human / I_{50} *Candida albicans*).

Table 2. Binding orientation data for inhibitors with *Candida albicans* and human DHFR enzyme

Compound	*C. albicans* DHFR					Human DHFR				
	E_{bind}[a]	$E_{interNC}$[b]	Rmsd[c]	H-bonds	ΔE[d]	E_{bind}	$E_{interNC}$	Rmsd	H-bonds	ΔE
1	−48.8	−7.86	1.60	3	3.22	−31.8	−3.60	0.96	2	5.43
2	−58.4	−12.44	1.47	3	3.29	−33.4	−3.25	1.12	–	1.44
3	−56.0	−8.85	1.48	2	2.99	−40.6	−3.88	0.95	2	8.40
4	−66.9	−8.25	1.25	3	1.91	−38.7	−4.10	0.88	2	2.79
5	−47.6	−7.35	0.92	3	4.14	−38.2	−3.97	0.79	1	2.64
6	−51.5	−7.90	1.13	3	2.31	−49.3	−4.23	0.74	3	2.77
7	−44.5	−7.48	1.21	1	2.91	−32.8	−6.67	0.63	1	2.00
8	−51.8	−8.32	1.23	3	3.50	−39.3	−4.34	0.94	4	4.46
9	−54.1	−8.21	1.20	3	3.70	−41.9	−5.20	0.77	3	6.34
10	−53.9	−7.36	1.48	3	4.00	−62.6	−5.84	0.58	3	12.2

[a] E_{bind} is energy of binding = $E_{complex} - (E_{enzyme} + E_{inhibitor})$ (kcal/mol).
[b] $E_{interNC}$ is interaction energy between compound and non-conserved amino acid residues of active site calculated from the EnergyAnalysis option within Discover 3.0 (kcal/mol).
[c] Rmsd is rms deviation of active site residues (all atoms) as compared to the refined crystal structure.
[d] ΔE is energy difference between energy of docked conformation and energy of global minimum energy conformation (kcal/mol).

energy minimized. The structure obtained from minimization was subjected to dynamics simulation at 300 K with equilibration for 10 ps, followed by simulation for 45 ps with a time step of 1 fs. Trajectory

frames were collected after every 200 steps. A distance dependent dielectric of 4.00 was used throughout the calculations. Nonbonded cutoffs were assigned using the cell multipole method. Trajectory data was analyzed using the Analysis module within Insight II. A set of trajectory frames were selected on the basis of potential energy, hydrogen bond interactions and strain energy for the compound for further studies. These selected trajectory frames were minimized until an rms of 0.001 kcal/mol Å was reached.

Solvation energy calculations

Electrostatic contributions to the solvation of the compounds were calculated using the Delphi module within Insight II. The Delphi program uses a finite differential solution to the non-linear Poisson–Boltzmann equation to calculate the electrostatic contribution to solvation energies. In the finite difference approximation, the molecule is mapped onto a three-dimensional cubical grid, with a grid spacing of unit size. The Poisson–Boltzmann equation was then used to calculate the potential at each grid point. For the interior of the protein a dielectric constant $\epsilon = 4$ was used, for the surrounding solvent a dielectric constant $\epsilon = 80$ was taken with an ionic strength of 0.145 M and for vacuum calculations the dielectric was set to 1.0. The solvent accessible surface area was calculated using a solvent probe radius of 1.4 Å. Energy calculations were performed using the CFF91 force field parameter set with the solvent dielectric set to 1.00, 4.00 and 80.0 to simulate vacuum, protein and aqueous environments. The electrostatic contribution to solvation free energy was calculated as the difference in the free energies in aqueous and vacuum environments while the electrostatic contribution to the desolvation free energy was taken as the difference between energies of protein and aqueous environments [19–21].

Results

5-Arylthio-2,4-diaminoquinazoline derivatives have been reported as inhibitors of *Candida albicans* dihydrofolate reductase. One of the most important factors in determining the use of these antifungal agents is their selectivity against fungal enzyme. A similar analysis of selectivity of DHFR inhibitors against bacterial and avian enzymes using QSAR methods has been reported [22].

A series of 10 compounds were selected based on I_{50} value, selectivity index and structural variations for studying the basis for selectivity through docking and dynamics simulations.

All the compounds were constructed using standard geometry and standard bond lengths. Lowest energy conformers were located by performing conformational search around two rotatable bonds.

For compound 1, two low energy conformers differing in torsion angle 1 (Table 1), with values of 67.8° and −135.5°, respectively, were selected. Active site minimization followed by dynamics and subsequent analysis showed that the conformation similar to one with starting torsion 1 equal to −135.5° is preferred for binding in the active site of fungal DHFR over the other conformer. This conformation is found to be orienting towards the Ile112 and Ile62 residues and away from the cofactor binding site. Similar orientations were observed for all other compounds. DHFR enzyme has been shown to exhibit multiple modes of substrate (dihydrofolate) binding [23]. These two conformations of dihydrofolate differ in their orientation of the pteridine ring in the active site. For the present series of compounds, two different orientations were studied for their interaction with the enzyme. From the docking studies it was observed that one of these orientations is favorable for binding. This orientation has also been found to be stable during dynamics simulations. Compounds used in the study are observed to exhibit only a single mode of binding.

Trajectory data from MD simulation was analyzed on the basis of the following parameters:

(a) Hydrogen bond interactions,
(b) Energy of binding $E_{binding} = E_{complex} - (E_{enzyme} + E_{compound})$,
(c) Conformational energy difference (ΔE) for the compound,
(d) Rms deviation of active site residues,
(e) Orientation of the inhibitor within the active site,
(f) Other interactions such as aromatic π-π stacking interactions, hydrophobic interactions.

Inhibition of DHFR is known to be mediated by hydrogen bond interactions between inhibitors and active site residues and these hydrogen bonds appear to be crucial for molecular recognition in all classes of DHFR inhibitors. Taking into consideration this factor we have used hydrogen bond interaction as one of the criteria for analysis.

Energy of binding was calculated for each compound after minimization of the selected trajectory frames, as the difference between the energy of the

complex and individual energies of the enzyme and compound [10]:

$$E_{binding} = E_{complex} - (E_{enzyme} + E_{compound})$$

Hydrogen bond interactions were used as one of the criteria for analysis of MD simulations. Literature suggests that the hydrogen bonds are the main recognition elements for DHFR–ligand systems. These hydrogen bonds were found to be stable throughout the minimization. In order to maintain uniformity during analysis, hydrogen bonds were held fixed and analysis was performed considering the other variables. Atoms of the 2,4-diaminoquinazoline nucleus involved in hydrogen bonding were fixed while others were not fixed. The conformational energy difference (ΔE) was calculated as the difference between the energy of the conformation within the active site and the energy of the global minimum conformation.

Interaction of each compound with both human and fungal DHFR enzyme was compared on the basis of interaction energy of each compound with the non-conserved amino acid residues of the active site. Interaction of each compound with the non-conserved amino acid residues of both the enzymes should ultimately govern the selectivity of these compounds for fungal DHFR. We have calculated the interaction energy of each compound with the non-conserved amino acid residues of both the enzymes using the Energy-Analysis option within Discover 3.0. Differences in the interaction energy reflect the differences in the inhibitory potency against fungal and human DHFR and, hence, will govern the selectivity for fungal DHFR.

Molecular docking and dynamics were performed on each compound with both the enzymes. The orientation for each compound discussed here represents the best orientation and is a representative of all possible interactions within the active site.

Compound 1

Compound 1 possesses a tertiary butyl group at the 4 position on the thioaryl ring and exhibits the highest activity among the series with better selectivity. Docking of compound 1 followed by dynamics simulations with fungal enzyme reveals that the t-butyl group accommodates itself in the active site around Ile112, resulting in the movement of active site residues. Compound 1 forms three hydrogen bonds (2-NH_2:Glu179-COOH; 2-NH_2:Tyr186-OH; 4-NH_2:Thr133-OH). The terminal butyl group is also involved in hydrophobic interactions with Ile112, Ile62 and this orientation is further stabilized by aromatic π–π interaction between Phe36 and the thioaryl ring. This results in a favorable orientation within the active site with energy of binding -48.8 kcal/mol and ΔE 3.22 kcal/mol. The binding orientation for compound 1 with fungal DHFR is depicted in Figure 1.

Interaction of 1 with human enzyme shows that this compound binds at a higher energy level with $\Delta E = 5.43$ kcal/mol. Compound 1 forms two hydrogen bonds (2-NH_2:Thr136-OH; 4-NH_2:Ile7) with the active site residues, resulting in energy of binding -31.8 kcal/mol. Compound 1 also exhibits interaction with the Val50, Val115, Thr38 and Ile7 residues. Binding of compound 1 with human DHFR results in the movement of active site residues, but this movement is restricted and results in a high energy for compound 1 after binding in the active site. Thus the differences in binding energy of compound 1 against two enzymes and ΔE values seems to govern the selectivity for compound 1. High conformational energy difference for human enzyme results in unfavorable binding with human DHFR, which is reflected in differences in binding energies. The docking orientation for compound 1 with human DHFR active site is shown in Figure 2.

Compound 2

Compound 2, with an n-hexyl side chain at the C4 position, shows poor inhibitory potency for fungal DHFR and low selectivity. Cyclization of this n-hexyl chain to a cyclohexyl group results in a high selectivity index for compound 3 (S.I. > 200). Interaction of compound 2 with fungal DHFR results in binding of the inhibitor at a site away from the actual binding site. The presence of an aliphatic hexyl side chain results in a tilted orientation within the active site and hence, poor binding. This orientation shows formation of three hydrogen bonds (2-NH_2:Tyr186-OH; 4-NH_2:Ala11; 4-NH_2:Thr133-OH). Compound 2 shows a binding energy of -58.4 kcal/mol and ΔE value of 3.3 kcal/mol. Although the presence of a highly lipophilic group might contribute to an overall better binding energy, the high entropic cost results in reduced binding and less inhibition. As in the case of fungal DHFR, the presence of an aliphatic group influences the binding with human enzyme. The inhibitor binds at the site away from the actual binding site. Thus, for compound 2 absence of strong hydrogen bonding interactions and a slightly different orientation within the active site results in poor inhibitory potency.

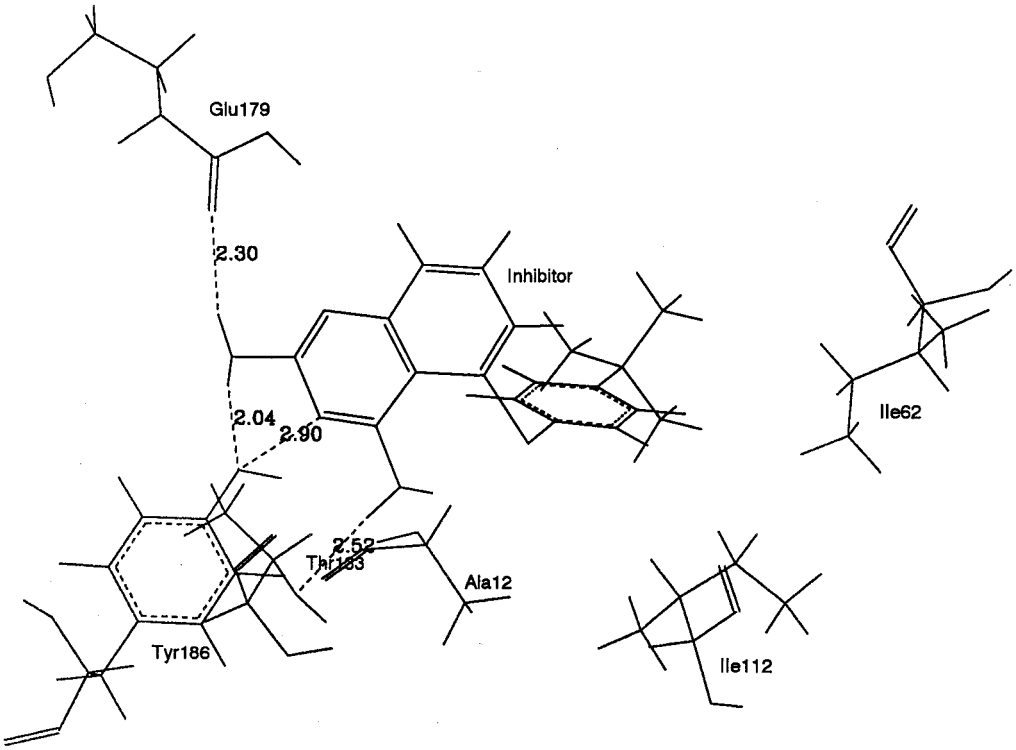


Figure 1. Binding orientation of compound 1 with *Candida albicans* DHFR active site residues with hydrogen bonds displayed.

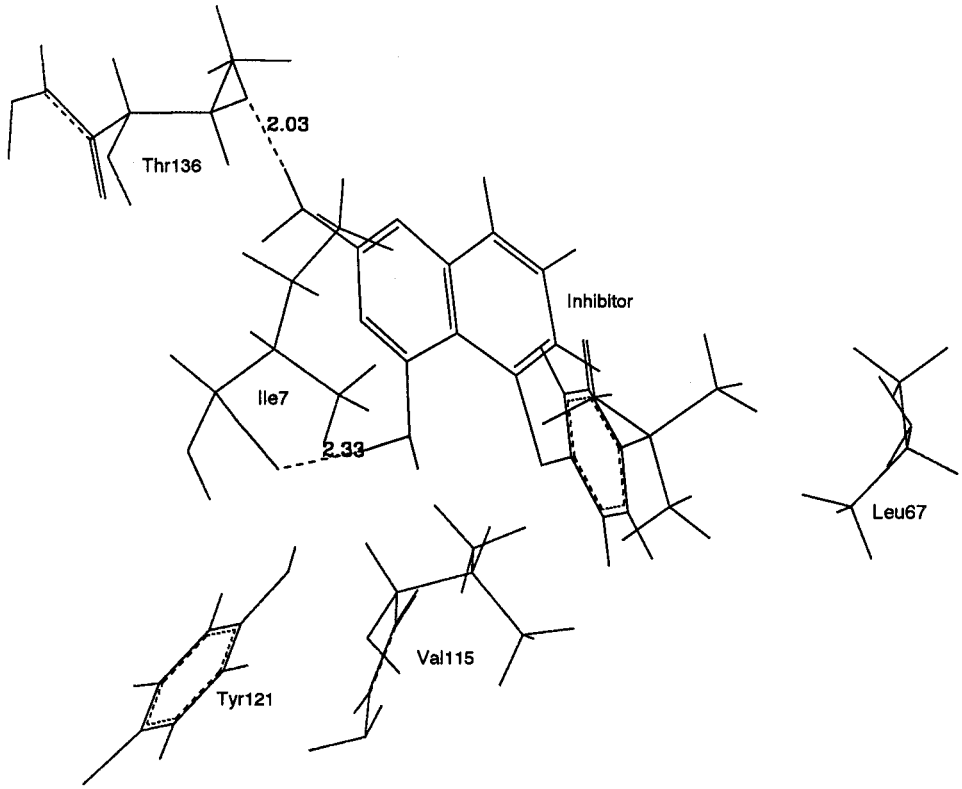


Figure 2. Binding orientation of compound 1 with human DHFR active site residues with hydrogen bonds displayed.

Compound 3

Incorporation of a flexible n-hexyl group into cyclohexyl increases the selectivity index to 200. Active site minimization followed by dynamics simulations results in favorable binding with *C. albicans* DHFR enzyme. The cyclohexyl group interacts with the hydrophobic residues of the active site. This orientation shows the presence of two hydrogen bonds between inhibitor and active site residues (N-1 quinazoline: Glu179-COOH; 2-NH_2:Tyr186-OH). The cyclohexyl ring in a chair conformation exhibits interactions with Ile112, Ile62, Pro63 and Leu69 in the hydrophobic pocket. For compound 3 it becomes important to understand the factors contributing towards the higher selectivity for fungal enzyme. The conformation adopted by compound 3 within the active site of human DHFR is at a higher energy (ΔE value of 8.4 kcal/mol). Another important observation is the absence of strong hydrogen bonds between compound 3 and the active site of the human enzyme. Because of the rigidity of the active site of the human enzyme, the inhibitor molecule gets 'trapped' or 'arrested' in a high energy state and this results in poor binding and inhibition. Comparison of energy of interactions between compound 3 and nonconserved active site residues shows the preferential selectivity against fungal enzyme: -8.85 kcal/mol; versus -3.88 kcal/mol against human enzyme.

Favorable binding with the fungal DHFR active site and unfavorable binding due to the high conformational energy difference ($\Delta E = 8.4$ kcal/mol) for human enzyme result in significant differences in binding energy for the two enzymes. These factors result in high selectivity for compound 3.

Compound 4

Compound 4, with a morpholine group at the C4 position, exhibits the highest selectivity index (540) among the series. The docking orientation shows a unique hydrogen bonding interaction between compound 4 and the fungal DHFR active site. Compound 4 forms three hydrogen bonds (N-1 quinazoline:Glu179-COOH; 2-NH_2:Tyr186-OH; morpholine oxygen:Arg28-NH) in its most favorable orientation with a high energy of binding, -66.9 kcal/mol, and a ΔE value of 1.91 kcal/mol. The hydrogen bond between arginine 28 and morpholine oxygen is unique and is observed to be stable during dynamics and minimization. The thioaryl ring is involved in a π–π interaction with Phe36. Analysis of the interaction with human enzyme reveals the absence of hydrogen bond interaction, similar to the fungal enzyme. Compound 4 exhibits two hydrogen bonds, with energy of binding -38.7 kcal/mol and $\Delta E = 2.79$ kcal/mol in human enzyme. The following factors make compound 4 highly selective against fungal DHFR:

(a) The morpholine ring at the C4 position interacts favorably with Arg28 of *C. albicans* enzyme. This interaction is absent in human enzyme.

(b) The favorable interaction with fungal enzyme is also reflected in a comparison of interaction energies with nonconserved residues of both fungal and human DHFR: fungal DHFR: -8.25 kcal/mol; human DHFR: -4.10 kcal/mol.

The high selectivity of compound 4 must be attributed to the presence of an additional hydrogen bonding interaction with Arg28 of fungal DHFR enzyme. This results in highly favorable binding, as evident from the binding energy and interaction energy of non-conserved residues with the fungal enzyme.

Compound 5

Compound 5 possesses a small, polar hydroxy group at C4 on the phenyl ring. Analysis of the interaction between compound 5 and fungal DHFR reveals that the –OH group is involved in hydrogen bond formation with Arg28. Although this interaction is unique, the hydrogen bond is not stable during the simulation and minimization. The favorable orientation involves three hydrogen bonds with energy of binding -47.6 kcal/mol and $\Delta E = 4.14$ kcal/mol (2-NH_2:Tyr186-OH; 4-NH_2:Thr133-OH; 4′-OH on thioaryl ring:Arg28-NH).

In the case of human enzyme, also the hydroxy group is involved in hydrogen bond formation, which is not stable during the simulations. The most favorable orientation shows a binding energy of -38.2 kcal/mol with $\Delta E = 2.64$ kcal/mol. It was observed that the hydroxy group interacts favorably with polar residues of the human enzyme such as Arg70, Thr38 and Thr39. The active site of human DHFR is more polar than the fungal enzyme, so that the hydroxy group interacts more favorably with these residues, resulting in better inhibition of human DHFR and thus in low selectivity. The high desolvation energy required for binding to the enzyme also contributes to reduced inhibition. The solvation and desolvation energies are presented in Table 3.

Compound 6

The presence of an –OCH_3 group at the 4 position on the phenyl ring results in a better selectivity index than

Table 3. Electrostatic contributions to solvation and desolvation free energies for compounds with fungal and human DHFR

Compound	E_{solv}[a]	E_{desolv}[b]	
		C. albicans DHFR	Human DHFR
1	−14.67	7.45	6.61
2	−13.92	6.20	5.33
3	−14.22	6.78	6.62
4	−14.97	6.96	7.58
5	−21.21	9.90	9.45
6	−14.62	7.04	7.44
7	−15.75	6.90	9.03
8	−17.66	9.10	8.21
9	−12.88	6.99	6.38
10	−14.91	7.60	7.64

[a] E_{solv} is the solvation energy of the lowest energy conformer in kcal/mol.
[b] E_{desolv} is the desolvation energy of the compound in kcal/mol.

its corresponding compound 5. Compound 6 interacts with formation of three hydrogen bonds with the fungal DHFR residues. The most favorable orientation results in an energy of binding of −51.5 kcal/mol and $\Delta E = 2.3$ kcal/mol, with the methoxy group interacting with the backbone of amino acid residues such as Ile112, Ile62, Phe36, Thr41 and Pro63. The orientation of compound 6 within the human DHFR active site shows the presence of three hydrogen bonds with a binding energy of −49.3 kcal/mol and $\Delta E = 2.77$ kcal/mol (N-1 quinazoline:Glu30-COOH; 2-NH_2:Thr136-OH; 4-NH_2:Ile7-CONH). The selectivity of compound 6 is also influenced by the nature of the active sites of the enzymes. A comparison of active sites (conserved as well as nonconserved residues) shows that the active site of human DHFR enzyme is more polar than the fungal enzyme. Thus the interaction of the methyl group in –OCH_3 with the active site of fungal DHFR results in better selectivity. This is also evident from a comparison of interaction energy with nonconserved residues: fungal DHFR: −7.90 kcal/mol; human DHFR: −4.23 kcal/mol.

The high selectivity of compound 6 results from favorable interaction with the fungal DHFR active site – hydrogen bond interaction with Arg28 -NH- and interaction of the methoxy group with nonpolar residues of the active site.

Compound 7

Compound 7, with a trifluoromethyl group, shows the lowest inhibitory potency in terms of I_{50} value. With this substituent it is expected that this compound would exhibit better inhibition. Interaction with the fungal DHFR shows the presence of only one hydrogen bond between the 4-NH_2 group and Thr133-OH. Compound 7 binds with fungal enzyme with energy of binding −44.5 kcal/mol and $\Delta E = 2.9$ kcal/mol. Observation of various orientations achieved shows the absence of strong hydrogen bonding interactions, resulting in low inhibitory potency for this compound. Interaction with the human enzyme results in an orientation with $\Delta E = 2.0$ kcal/mol and energy of binding −32.8 kcal/mol, and this orientation shows the presence of one hydrogen bond. The desolvation energy required for binding to human enzyme is high (∼9.0 kcal/mol), resulting in low inhibition.

Compound 8

Compound 8 is similar to compound 1, with an amino group at the C_6 position on the quinazoline ring system. Compound 8 shows low inhibitory potency and a low selectivity index as compared to compound 1. Active site minimization and subsequent MD simulations show that the inhibitor is involved in extensive and strong hydrogen bonding interactions with the *C. albicans* DHFR active site. The amino group at C6 was observed to be involved in hydrogen bond formation, but this bond was not stable during the simulation. The favorable orientation shows a binding energy value of −51.8 kcal/mol with formation of three hydrogen bonds (2-NH_2:Tyr186-OH; 4-NH_2:Ala11; 4-NH_2:Thr133-OH). This binding with the enzyme occurs at a higher energy level, with $\Delta E = 3.5$ kcal/mol.

Interaction of compound 8 with human DHFR results in a strong hydrogen bonding interaction and the favorable docking model shows the presence of four hydrogen bonds (2-NH_2:Thr136-OH; 2-NH_2:Val8; N-1 quinazoline:Glu30-COOH; 4-NH_2:Ile7). This results in a binding energy of −39.3 kcal/mol and $\Delta E = 4.46$ kcal/mol. The presence of a highly polar amino group results in a high energy of desolvation for compound 8. This would result in reduced binding and low inhibitory potency.

Compound 9

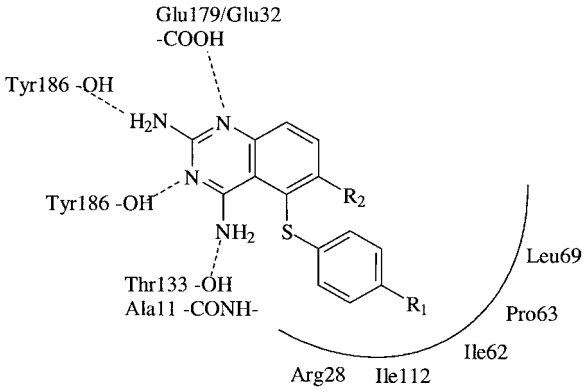
Compound 9 possesses an isobutyloxy group at the C6 position on the quinazoline nucleus and exhibits equivalent potency as compared to compound 1.

Analysis of its interaction with fungal DHFR shows that the conformation achieved by compound 9 within the active site is at higher energy and results in a high ΔE value. During MD simulations, the oxygen atom of the isobutoxy group is involved in hydrogen bond formation with Arg28 and this was found to be stable throughout the simulations. This becomes an additional recognition point for compound 9, which exhibits three hydrogen bonds (N-1 quinazoline:Glu179-COOH; 4-NH_2:Tyr186-OH; -O-isobutoxy:Arg28-NH). The isobutyl group interacts with hydrophobic amino acids like Tyr21, Val10, and Pro63. This orientation shows an energy of binding of -54.1 kcal/mol with $\Delta E = 3.7$ kcal/mol. These strong and stable interactions seem to contribute to overall better binding and better inhibition of fungal enzyme. Although compound 9 exhibits three hydrogen bonds, it also shows a high ΔE value of 6.3 kcal/mol for human enzyme. The most favorable orientation shows the presence of three hydrogen bonds (2-NH_2:Thr136-OH; 2-NH_2:Val8; 4-NH_2:Ile7) with energy of binding $= -41.9$ kcal/mol. Non-specific hydrophobic interactions between the isobutyl group and active site residues like Leu22, Pro61, Phe31 and Ile60 also contribute towards better inhibition of human enzyme and hence, low selectivity.

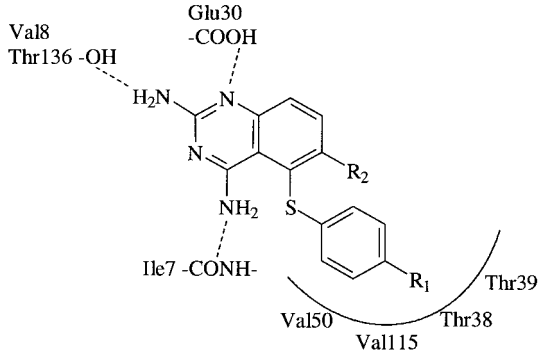
Compound 10

The trimethoxy substituents at the 3, 4 and 5 positions on the thioaryl ring impart high potency and better selectivity (selectivity index = 110). Docking followed by molecular dynamics simulation results in an active site orientation which shows a large number of hydrogen bonds with the fungal DHFR active site. The best docking model shows three hydrogen bonds (2-NH_2:Tyr186-OH; N-1 quinazoline:Glu32-COOH; 5′-OCH_3:NADPH-$CONH_2$). This orientation results in an energy of binding of -53.9 kcal/mol with $\Delta E = 4.0$ kcal/mol. The methyl groups on the phenyl ring exhibit strong hydrophobic interactions with Leu69, Pro70, Ile62, Ile112, Pro63, Phe66, etc.

Interaction of compound 10 with human DHFR reveals that the binding within the active site occurs at a very high energy with $\Delta E = 12.2$ kcal/mol. This orientation results in formation of four hydrogen bonds with binding energy $= -62.6$ kcal/mol. Methyl groups on the phenyl ring appear to be exposed to polar residues such as Asn64, Thr56, etc. This factor, along with a high conformational energy ΔE value in case of human enzyme results in better selectivity for fungal enzyme by this compound.



Candida albicans DHFR



Human DHFR

Figure 3. Schematic representation of interaction between inhibitor compounds with *Candida albicans* DHFR and human DHFR active site.

Discussion

Docking studies and subsequent molecular dynamics simulations for 5-arylthio-2,4-diaminoquinazolines as *C. albicans* DHFR inhibitors have resulted in a new insight into the factors responsible for their potency and selectivity. A schematic diagram of all the interactions between these compounds and fungal and human DHFR is presented in Figure 3.

As with other classes of DHFR inhibitors, molecular recognition and subsequent interaction with enzyme active site residues occur mainly through hydrogen bonding interactions. These specific hydrogen bonds appear to be anchoring points for these compounds. Binding affinity and inhibitory potency (I_{50}) are mainly influenced by these hydrogen bonding interactions. All such interactions between compounds and DHFR enzyme (fungal and human) are summarized in Table 3. Compounds 1, 3, 6 and 9 exhibit-

Table 4. Important hydrogen bonding interactions between compounds and *C. albicans* and human DHFR enzyme active site residues

Group/atom of compounds involved in interaction	Fungal DHFR residue	Human DHFR residue
N-1 quinazoline	Glu179 -COOH	–
	Glu32 -COOH	Glu30 -COOH
2-NH_2 group	Tyr186 -OH	Val8 -CONH-
		Thr136 -OH
N-3 quinazoline	Tyr186 -OH	–
4 -NH_2	Thr133 -OH	Ile7 -CONH-
	Ala11 -CONH-	
Morpholine oxygen in 4	Arg28 -NH-	–
C4′ -OH in 5	Arg28 -NH-	–
C4′ -OCH_3 in 6	Arg28 -NH-	–
Isobutoxy group in 9	Arg28 -NH-	–

ing these interactions possess high inhibitory potency (I_{50}). All these compounds form three to four specific hydrogen bonds with the active site residues. Binding of these compounds within the active site of *C. albicans* DHFR results in a large movement of active site residues so as to accommodate these compounds. This is evident from the large rms deviation of active site residues of the enzyme. The active site residues surrounding the Ile62 and Ile112 residues seem to accommodate the bulky substitution on the phenyl ring. The active site is flexible and is able to accommodate groups such as t-butyl, cyclohexyl, etc. Apart from the hydrogen bond interactions specific to the quinazoline nucleus, substituents on the thioaryl ring are also involved in hydrogen bond formation. Hydroxy and methoxy groups in compounds 5 and 6 are involved in hydrogen bond formation with Arg28-NH-groups, while morpholine oxygen in compound 4 is also involved in hydrogen bond formation with Arg28. Amino and isobutyloxy groups in compounds 8 and 9 form hydrogen bonds with Arg28. Apart from this hydrogen bond formation, hydrophobic interactions seem to contribute significantly to overall binding. These interactions are mainly observed between substituents on the phenyl ring such as t-butyl, cyclohexyl, methoxy, and active site residues like Leu69, Ile62, Ile112, Tyr41 and Pro63. Aromatic π-π stacking interaction between the thioaryl ring and Phe36 also contributes towards the binding. Compounds with high selectivity exhibit favorable binding with fungal DHFR active site residues because of these various interactions. Favorable interaction of these compounds is also reflected in binding energy values.

In case of human DHFR, hydrogen bonds formed between inhibitor and enzyme active site residues are important for recognition and inhibition. But in case of the human enzyme, the active site appears to be very rigid and movement of active site residues on binding of the compound is less as compared to fungal enzyme. This rigidity of the active site is evident from the low rms deviation of active site residues. Since the fungal DHFR active site is flexible, binding of these compounds within the active site results in a large movement of active site residues. The rmsd values for fungal DHFR active site residues (all atoms) range from 0.92 to 1.60. In case of the human enzyme, the active site is very rigid and binding of the compounds does not result in any large movement, as evident from the relatively low rmsd of active site residues, in the range of 0.58 to 1.12. Thus compounds exhibit very high energy when bound inside the active site and, consequently, high ΔE values for compounds in case of human DHFR. High conformational energy differences result in unfavorable interaction, which is evident from low values of binding energy. This effect is more pronounced in compounds with bulky substituents on the phenyl ring. Thus the nature and flexibility of the active site, as evident from rmsd on binding of compounds, plays an important role in determining the selectivity of the compounds. The energy difference between the conformation in the active site and the minimum energy conformation acts as a negative driving force for binding of inhibitors to

human enzyme. For compounds 1, 3 and 4, the ΔE value is higher for human enzyme compared to fungal enzyme. Moreover, the fact that compounds 2, 5 and 7, which show low conformational energy difference (ΔE values) for human DHFR, exhibit better activity against human enzyme (hence, low selectivity) also supports this fact. For compound 6, the methoxy group is involved in favorable interaction with the active site residues of fungal enzyme and exhibits better selectivity. This favorable interaction is supported by the large differences in binding energy for the two enzymes. For compound 5, the presence of a polar hydroxyl group results in a more favorable interaction with human DHFR active site polar residues and thus in better activity and low selectivity. It is observed that the solvation and desolvation phenomenon plays an important role in determining the inhibitory potency of compounds containing polar substituents.

In general, it is observed that the hydrogen bonding and hydrophobic interactions mainly contribute towards the activity of these *C. albicans* DHFR inhibitors. The active site of fungal DHFR is flexible as compared to the human enzyme active site and hence, compounds with bulky and non-polar groups induce large movement of active site residues. In case of human DHFR, binding of the compound within the active site occurs at a higher energy state. Thus the presence of bulky functional groups results in high selectivity against fungal enzyme. In summary, the nature of the active sites and their effect on the binding conformations of the inhibitors influence the inhibitory potency and selectivity of these inhibitors.

The present study was performed on a selected set of compounds with different inhibitory potencies and selectivity indexes. Although the compounds are similar in structure, due to some specific interactions with the enzyme, different energy terms or parameters such as binding energy, interaction energy with non-conserved residues, conformational energy difference (ΔE) and hydrogen bonding interactions were calculated and analyzed. It has been observed that in the present series of compounds some specific parameters are responsible for favorable interaction of specific compounds with the enzyme and hence, different energy terms or parameters explain the selectivity of the compounds. However, major factors contributing to the selectivity of the present series of compounds include binding energy, conformational energy difference (ΔE) and specific hydrogen bonding and hydrophobic interactions. Results from the present study are informative in a qualitative manner. In the present study no attempt has been made to quantitate the results. Such a quantitative study would require a larger set of compounds and an accurate modeling protocol. It is not part of this study to include any common scoring function for comparison of various energy terms and a quantitative equation correlating these terms with biological activity.

A number of literature reports are available concerning computational aspects of DHFR–ligand complexes. The present study is unique in the sense that a comparison of interaction of DHFR inhibitors with fungal and human enzymes has been made. It attempts to understand the structural factors responsible for potency and selectivity of DHFR inhibitors. Potency and selectivity of inhibitors have an important role during clinical application of these agents as antifungals. Results from this selectivity analysis can be utilized in the design of agents with no inhibitory effect on human enzyme and, consequently, less side effects to the human host. The present study attempts to address this important question of selectivity against a fungal enzyme target for which a similar enzyme is present in the human host.

Conclusions

A series of 5-(arylthio)-2,4-diaminoquinazolines as inhibitors of *Candida albicans* DHFR were studied by docking and dynamics simulations with the aim to reveal the structural factors responsible for their inhibitory potency and selectivity. Analysis of dynamics trajectories shows that the large hydrophobic substituent at the C4 position on the thioaryl ring orients towards the Ile62 and Ile112 residues in the fungal DHFR enzyme. Inhibitory potency and selectivity of the compounds is governed by the favorable binding and specific hydrogen bonding interactions within the active site of fungal DHFR. The fungal DHFR active site is flexible and can accommodate bulky groups on thioaryl rings. The human enzyme active site is very rigid and hence, the compounds bind in their high energy conformation. This results in unfavorable binding with the human DHFR enzyme and this is evident from differences in the binding energy values. The differences in binding orientation and ΔE value govern the selectivity of these inhibitors. The information generated from this study of potency and selectivity should be useful for future work in this area of research.

Acknowledgements

The authors thank the University Grants Commission, New Delhi for financial support under the Special Assistance Program and COSIST. One of the authors (V.M.G.) thanks the University Grants Commission (UGC) for a Senior Research Fellowship. V.M.G. also thanks Mr. Santosh Kulkarni for useful suggestions.

References

1. Kuyper, L.F., In Perun, T.J. and Propst, C.L. (Eds.) Computer-Aided Drug Design Methods and Applications, Marcel Dekker Inc., New York, NY and Basel, 1989, pp. 327–369.
2. McCormack, J., In Hansch, C. and Sammes, P.G. (Eds.) Comprehensive Medicinal Chemistry, Vol. 2., Pergamon Press, London, 1990, pp. 271–298.
3. Volz, K.W., Matthews, D.A., Alden, R.A., Freer, S.T., Hansch, C., Kaufman, B.T. and Kraut, J., J. Biol. Chem., 257 (1982) 2528.
4. Matthews, D.A., Alden, R.A., Bolin, J.T., Filman, D.J., Freer, S.T., Hamlin, R., Hol, W.G.J., Kisluik, R.L., Pastore, E.J., Plante, L.T., Xuong, N. and Kraut, J., J. Biol. Chem., 253 (1978) 6946.
5. Bolin, J.T., Filman, D.J., Matthews, D.A., Hamlin, R.C. and Kraut, J., J. Biol. Chem., 257 (1982) 13650.
6. Whitlow, M., Howard, A.J., Stewart, D., Hardman, K.D., Kuyper, L.F., Baccanari, D.P., Fling, M.E. and Tansik, R.L., J. Biol. Chem., 272 (1997) 30289.
7. Chan, J.H., Hong, J.S., Kuyper, L.F., Baccanari, D.P., Joyner, S.S., Tansik, R.L., Boytos, C.M. and Rudolph, S.K., J. Med. Chem., 38 (1995) 3608.
8. Kuyper, L.F., Baccanari, D.P., Jones, M.L., Hunter, R.N., Tansik, R.L., Joyner, S.S., Boytos, C.M., Rudolph, S.K., Knick, V., Robert Wilson, H., Marc Caddell, J., Friedman, H.S., Comley, J.C.W. and Stables, J.N., J. Med. Chem., 39 (1996) 892.
9. Inoue, A., Kawai, T., Wakita, M., Iimura, Y., Sugimoto, H. and Kawakami, Y., J. Med. Chem., 39 (1996) 4460.
10. Yamamoto, Y., Ishihara, Y. and Kuntz, I.D., J. Med. Chem., 37 (1994) 3141.
11. Hariprasad, V. and Kulkarni, V.M., J. Mol. Model., 3 (1997) 443.
12. Rastelli, G. and Costantino, L., Bioorg. Med. Chem. Lett., 8 (1998) 641.
13. Kyle, D.J., Chakravarty, S., Sinsko, J.A. and Stormann, T.M., J. Med. Chem., 37 (1994) 1347.
14. Furet, P., Caravatti, G., Lydon, N., Priestle, J.P., Sowadski, J.M., Trinks, U. and Traxler, P., J. Comput.-Aided Mol. Design, 9 (1995) 465.
15. Winter, H.D. and Herdewijn, P., J. Med. Chem., 39 (1996) 4727.
16. Wang, S., Karanietz, M.G., Blumberg, P.M., Marquez, V.E. and Milne, G.W.A., J. Med. Chem., 39 (1996) 2541.
17. Insight II 97.0 Molecular Modeling software is available from Molecular Simulations Inc., San Diego, CA.
18. Discover 3.0.0 User Guide, October 1995, Molecular Simulations Inc. San Diego, CA.
19. Gilson, M.K. and Honig, B., Proteins, 4 (1988) 7.
20. Kulkarni, S.S. and Kulkarni, V.M., J. Chem. Inf. Comput. Sci., 39 (1999) 1128.
21. Hariprasad, V. and Kulkarni, V.M., J. Mol. Recogn., 9 (1996) 95.
22. Selassie, C.D., Gan, W., Kallander, L.S. and Klein, T.E., J. Med. Chem., 41 (1998) 4261.
23. Birdsall, B., Feeney, J., Tendler, S.J.B., Hammond, S.J. and Roberts, G.C.K., Biochemistry, 28 (1989) 2297.