J Mol Model (2011) 17:657–667
DOI 10.1007/s00894-010-0756-y

ORIGINAL PAPER

Computer-aided molecular design of 1H-imidazole-2, 4-diamine derivatives as potential inhibitors of *Plasmodium falciparum* DHFR enzyme

Legesse Adane · Prasad V. Bharatam

Received: 4 March 2010 / Accepted: 12 May 2010 / Published online: 5 June 2010

Abstract Design and discovery of new potential inhibitors of *Plasmodium falciparum* dihydrofolate reductase (PfDHFR), equally active against both the wild-type and mutant strains, is urgently needed. In this study, a computer-aided molecular design approach that involved ab initio molecular orbital and density functional theory calculations, along with molecular electrostatic potential analysis, and molecular docking studies was employed to design 15 1H-imidazole-2,4-diamine derivatives as potential inhibitors of PfDHFR enzyme. Visual inspection of the binding modes of the compounds demonstrated that they all interact, via H-bond interactions, with key amino acid residues (Asp54, Ileu/Leu164, Asn/Ser108 and Ile14) similar to those of WR99210 (**3**) in the active site of the enzymes used in the study. These interactions are known to be essential for enzyme inhibition. These compounds showed better or comparable binding affinities to that of the bound ligand (WR99210). In silico toxicity predictions, carried out using TOPKAT software, also indicated that the compounds are non-toxic.

Keyword *Plasmodium falciparum* · Dihydrofolate reductase enzyme inhibitor · 1H-imidazole-2,4-diamine derivatives · Computer-aided molecular design

L. Adane · P. V. Bharatam (✉)
Department of Medicinal Chemistry,
National Institute of Pharmaceutical
Education and Research (NIPER),
S.A.S. Nagar-160 062,
Mohali, Punjab, India
e-mail: pvbharatam@niper.ac.in

Introduction

The dihydrofolate reductase domain of the bifunctional *Plasmodium falciparum* enzyme dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) is one of the validated targets in malaria chemotherapy. PfDHFR catalyses the nicotinamide adenine nucleotide phosphate (NADPH) dependent reduction of dihydrofolate (DHF) to tetrahydrofolate (THF), which is essential for DNA synthesis in the parasitised cell [1–3]. This enzyme has also been recognised as a specific target for common antimalarial antifolates such as cycloguanil (**1**) and pyrimethamine (**2**) (Fig. 1). However, due to the emergence and spread of drug-resistant strain of the parasite, the therapeutic value of these drugs has been compromised in many parts of the world, particularly in sub-Saharan Africa, Latin America, southern Asia and Oceania [4].

Homology modeling [5–7] and X-ray crystallographic [8, 9] studies have clearly indicated that accumulation of genetic mutation at one or more amino acid residues in the active site of the PfDHFR enzyme is responsible for resistance of the parasite toward antifolate-based antimalarial drugs. For instance, *P. falciparum* parasite harbouring PfDHFR mutations Ala16Val + Ser108Thr showed resistance to **1** but was sensitive to **2**, whereas a single mutation Ser108Asn causes resistance to **2** that is also enhanced by additional mutations Asn51Ile + Cys59Arg. On the other hand, cross-resistance occurs to **1** and **2** in a *P. falciparum* parasite strain carrying DHFR enzyme with mutations at amino acid residues 51, 59, 108 and 164 [5–7, 10, 11]. In all cases, these mutations cause a steric clash that displaces the drugs from their optimal orientations in their interaction with key amino acid residues such as Asp54, which is crucial for inhibitor binding. This displacement, in turn, ultimately results in a significant loss of affinity and

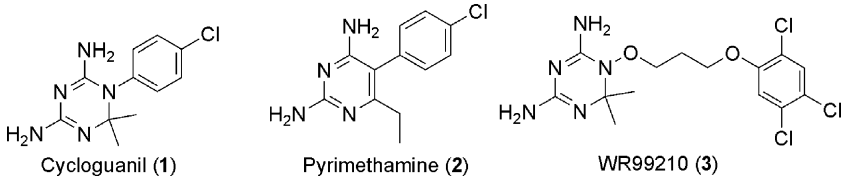


Fig. 1 Chemical structures of cycloguanil (**1**), pyrimethamine (**2**) and WR99210 (**3**)

consequently the enzyme inhibitory activities of these drugs. In the parasite harboring Ala16Val + Ser108Thr mutated DHFR enzyme, it was found that one of the methyl groups at the 6-position of **1** experiences a severe steric interaction with Val16 that decreases the binding affinity of the inhibitor. In the case of a parasite with a DHFR enzyme with multiple mutations, steric clashes of the para-Cl groups of both **1** and **2** with Asn108 (mutated from Ser108) result in the loss of inhibitory activities of the drugs [6].

Several structure-activity relationship (SAR) studies [12–15] and reviews [15–17] have helped increase our understanding of the molecular level mechanisms of *P. falciparum* resistance to antifolates, and also in the design of antimalarial agents. Based on the steric constraint hypothesis, different research groups have employed rational drug design approaches to design compounds that could avoid steric clashes in the active sites, particularly with the amino acid residue at 108, of the mutant parasite. McKie et al. shifted the para-Cl of **2** to the meta-position to design several analogs exhibiting 30-fold higher activity against Cys59Arg + Ser108Asn double mutant PfDHFR enzyme as compared to the parent compound [18]. Similarly, Sardarian et al. designed and synthesised several meta-substituted analogs of **2** that showed generally better activities than the parent compound against the quadruple mutant PfDHFR enzyme [19]. Rastelli et al. also reported analogs of WR99210 (**3**), replacing one of the methyl groups at the 6-position with a hydrogen atom. This resulted in compounds with better inhibitory activities against the Ala16Val + Ser108Thr mutant PfDHFR enzyme as compared to **3** [6]. Experimental observations by Kamchonwongpaisan et al. [20] and Yuthavong et al. [21] also support the above hypotheses.

It is well established that compounds that are more flexible than the common antifolates (e.g., **1** and **2**) can effectively inhibit mutant PfDHFR enzymes [8, 11, 22]. A third-generation antifolate, **3**, is a typical example of a compound designed to avoid steric clash with Asn108 of the mutant PfDHFR enzyme. Inhibitory activity tests indicated that **3** has similar activities (in nM ranges) against *P. falciparum* parasites that harbour wild-type and multiple mutant DHFR enzymes [11]. Because of its flexible side chain, **3** adopts a conformation that fits into the active site of the mutant enzyme, and is also devoid of bulky groups in proximity to Asn108 and thus effectively inhibits the mutant parasite. Unfortunately, because of its gastrointestinal intolerance and poor bioavailability in animal studies, this compound has not advanced to the clinical trial stage [23, 24], although it is still being used as a model compound for designing competitive PfDHFR enzyme inhibitors of flexible nature [25].

A number of reports are available on the successful application of computer-aided molecular design (CAMD) approaches to the design of antimalarial agents [26–29]. Many such reports have shown promising results. However, no successful drug has come to the market to date. Therefore, there is an urgent and unmet need for new drugs to combat malaria. Here, we present 1H-imidazole-2,4-diamine derivatives designed by employing CAMD approach as potential inhibitors of PfDHFR. To the best of our knowledge, these compounds have not previously been designed as potential inhibitors of PfDHFR enzyme. The CAMD approaches used in this study include ab initio molecular orbital (MO) and density functional theory (DFT) calculations, as well as molecular electrostatic potential (MESP) calculations, molecular docking and in silico property predictions. The results indicate that the designed compounds show properties comparable to those of **3**. Moreover, the 1H-imidazole-2,4-diamine scaffold is commercially available, suggesting that the designed compounds could be synthetically accessible (feasible). Thus, in the following sections of this paper, we discuss the details of the CAMD approaches used to design the title compounds.

Materials and methods

Ab initio MO [30, 31] and DFT [32, 33] calculations were carried out using the GAUSSIAN03 package [34]. Complete optimisations on the isomers of the 1H-imidazole-2,4-diamaine and 1H-imidazole-2,5-diamaine scaffolds were performed using B3LYP [35–37] method with the 6-31+G* basis set. Frequencies were also computed analytically for the optimised isomers to characterise each stationary point as a minimum or a transition state, and also to estimate the zero point vibrational energies (ZPE). The calculated ZPE values (at 298.15K) were scaled by a factor of 0.9806 [38]. The conductor-like polarizable continuum (CPCM) solvent model was employed during energy calculations in water medium [39]. Geometry optimisations were carried

out using the B3LYP/6-31+G* level for solvent effect calculations.

MESP values were calculated on the B3LYP/6-31+G* optimized geometries of the isomers of the 1H-imidazole-2,4-diamaine scaffold using SPARTANPro software [40]. The colour-coded surface provides a location of the positive (deepest blue, most positive) and negative (deepest red, most negative) electrostatic potentials. The regions of positive charge indicate relative electron deficiency (estimated as a function of the repulsion experienced by a positively charged test probe), and regions of negative potential indicate areas of excess negative charge (estimated as a function of the attractive force experienced by a positively charged test probe).

Glide v4.5 was used for molecular docking studies [41, 42]. During the protein preparation step, only chain **A** and cofactor NDP610 of the bifunctional PfDHFR-TS enzyme were retained. All water molecules and the rest of the chains (**B**, **C** and **D**) were removed. A radius of 15 Å was selected for the active site cavity during receptor grid generation [41, 42]. Molecules were drawn in SYBYL7.1 [43] and were imported into a glide window and subsequently converted into Maestro format using the LigPrep module of Glide. The flexible docking option and XP mode were used in all calculations. The reproducibility of the docking calculation was evaluated by docking the bound ligand into the prepared active sites. The output was set to give ten docking poses/ligand and default values were used for other parameters [43]. The results indicated the top scoring conformations of the bound ligand to have root-mean-square-deviation (RMSD) values of 0.374 and 0.489 Å for the wild-type and quadruple mutant PfDHFR enzymes, respectively, demonstrating that the prepared protein could be used for docking studies of other compounds. The binding modes and interactions of the study compounds with key amino acid residues in the active site of the enzymes were analysed by visual inspection. Toxicity and other related parameters were predicted using TOPKAT software implemented in Accelrys Discovery Studio 2.5 [44]. The reference compound (**3**) was also included as control.

Results and discussion

The CAMD approach is becoming common practice in drug discovery and development programs for several reasons. For example, it is (1) fast, (2) more economical compared to in vitro and in vivo biological (high-through put screening) tests, and (3) it enables the researcher to predict most of the physico-chemical and biological properties of a compound before its synthesis [45–47]. As a continuation of our effort to design potential antimalarial agents targeting wild-type and mutant varieties of PfDHFR enzymes, we present the design of 1H-imidazole-2,4-diamine derivatives. The compounds were designed by employing different CAMD methodologies in an integrated fashion as described below.

Close inspection of the interactions and the binding mode of **3** [8, 9], together with the results of our recent study [25], indicated that ligands that satisfy the following three criteria could be potential inhibitors of PfDHFR enzymes: (1) hydrogen bond donor (HBD) head groups that can form H-bond interactions with Asp54, Ile14 and Ile/Leu164; (2) hydrophobic aromatic tails bearing one or more substituent groups such as Cl and OMe (these tails occupy the hydrophobic pocket of the active site to enhance inhibitory activities); and (3) a linker unit between the HBD head groups and the hydrophobic tails that provides flexibility to the ligand in order to avoid potential unfavourable steric clashes with Asn108 in the active site of the mutant PfDHFR enzyme. Figure 2 shows a schematic representation of important regions in the active site of PfDHFR enzymes required for interactions with long and

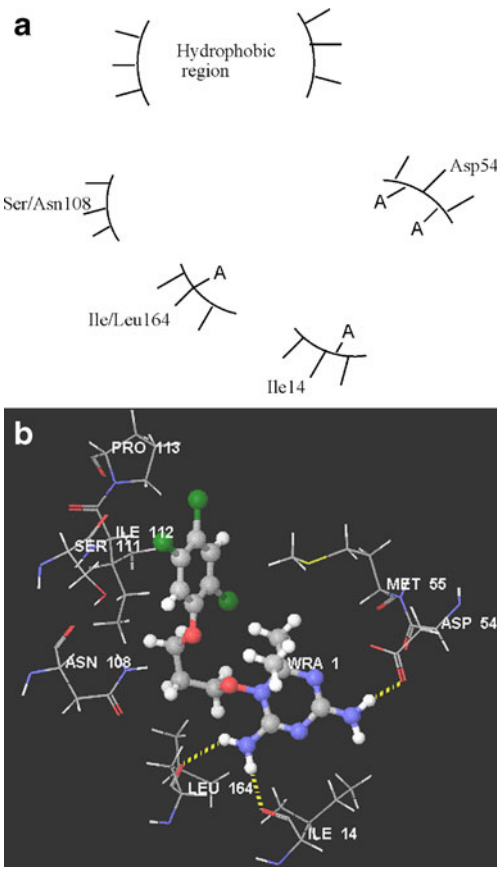


Fig. 2 Schematic representation of **a** important interaction regions in the active sites of *Plasmodium falciparum* dihydrofolate reductase (PfDHFR) enzymes, and **b** the binding mode of **3** in the active site of the quadruple mutant PfDHFR enzyme. *A* Hydrogen bond acceptor amino acid residues

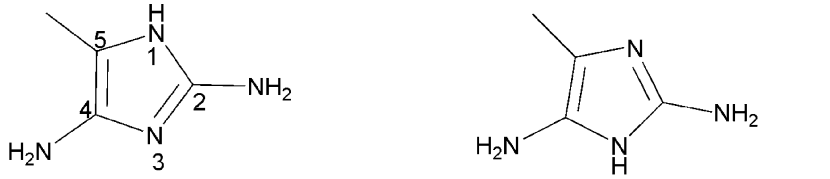


Fig. 3 Chemical structures of 5-methyl-1H-imidazole-2,4-diamine (**4**) and 4-methyl-1H-imidazole-2,5-diamine (**5**)

flexible ligands similar to **3** (Fig. 2a), and the binding mode of **3** in the active site of the quadruple mutant PfDHFR enzyme (Fig. 2b). In this study, the 1H-imidazole-diamine moiety was used as the HBD head group, replacing the 2,4-diaminopyrimidine and/or 1,3,5-triazine moieties of the known antifolates (Fig. 1). This moiety can be represented by two isomers: the 1H-imidazole-2,4-diamine and 1H-imidazole-2,5-diamine moieties. Therefore, an ab initio MO and DFT computational study was carried out in order to determine the most stable (preferred) isomer. The isomers used in this computational study were 5-methyl-1H-imidazole-2,4-diamine (**4**) and 4-methyl-1H-imidazole-2,5-diamine (**5**) (Fig. 3). The results demonstrated that isomer **4** is marginally more stable than isomer **5** in both gas phase and aqueous medium by 1.23 and 1.44 kcal mol^{-1}, respectively (Table 1). The greater stability of isomer **4** can be attributed to the absence of the 1,3-allyl-type interaction across C2–N3–N4 as observed in biguanide derivatives [48, 49]. Thus, several compounds that satisfy the above mentioned three criteria were proposed based on the 1H-imidazole-2,4-diamine moiety of isomer **4**. H-bond interactions of ligands with the key amino acid residues of PfDHFR enzyme are crucial for inhibitory activity. Therefore, 1H-imidazole-2,4-diamine derivatives are expected to form H-bond interactions with the H-bond receptor residues (at 14, 54 and 164) of the PfDHFR enzyme that are comparable to those of the known antifolates (**1**–**3**) [6, 8, 9]. This was demonstrated by the observed distances between the hydrogen atoms of the amino groups of the 1H-imidazole-2,4-diamine core of isomer **4**, which are similar to those of **2** and **3** (Fig. 4). This suggested that the 1H-imidazole-2,4-diamine core could form optimal H-bond interactions with the enzyme similar to that of the 2,4-diaminopyrimidine moiety of **2** (b) and/or 1,3,5-triazine moiety of **3**.

For any molecule to elicit the pharmacological activity, molecular recognition during ligand-receptor interactions is of a paramount importance. This is governed by the stereo electronic effects of ligands. Thus, a study of the three-dimensional MESP and its gradient plots becomes important in understanding the complementarity of the electronic surface of the ligand and the active site. MESP analysis provides information regarding the surface properties of ligands, which are crucial for non-covalent interactions with amino acid residues of protein targets. Colour-coded plots of MESP can be drawn to visually model the electrostatic surface of ligands (drugs), which ultimately helps in designing a drug that is complementary to a particular target [49–51]. Thus, MESP analysis was carried out to investigate the similarities of the 1H-imidazole-2,4-diamine scaffold and the 2,4-diaminopyrimidine moiety of the known antifolate drugs such as **2**. The results indicated that the MESP contour of the 1H-imidazole-2,4-diamine scaffold was similar to that of the N1-protonated 2,4-diaminopyrimidine core of **2** (Fig. 5). This also suggested that, like the protonated antifolates, the 1H-imidazole-2,4-diamine core could form bidentate H-bond interactions with both the oxygen atoms of the carbonyl group of Asp54 (of PfDHFR enzyme). This interaction is believed to increase the binding affinities and inhibitory activities of antifolate-based antimalarial agents [52]. Therefore, any surface (target) complementary to pyrimethamine core or the HBD head groups of the known antifolates is expected to show similar complementarity to 1H-imidazole-2,4-diamine head group/derivatives. Thus, several compounds were proposed based on this head group as potential inhibitors of PfDHFR enzyme. Figure 6 shows the chemical structures of the designed compounds. As can be seen from their structures, all these compounds fulfill the three criteria mentioned above [25]. That is, they possess an aromatic tail, flexible linker units and HBD head groups. Moreover, the compounds satisfy the drug-like properties suggested by Lipiniski's rule-of-five, which states that a compound with molecular weight <500 Da, number of H-bond donors (the sum of NHs and OHs) <5, number of H-bond acceptors (the sum of Ns and Os) <10 and logP <5 is considered as a drug-like compound [53]. As given in Table 2, most of these properties are within the acceptable ranges, suggesting that all the designed compounds possess drug-like

Table 1 Zero point vibrational energies (ZPE) corrected relative energies (RE; kcal mol^{-1}) of isomers **4** and **5**

Compound	RE[a]	RE[b]
4	0.00	0.00
5	1.23	1.44

[a] In gas-phase

[b] In aqueous phase

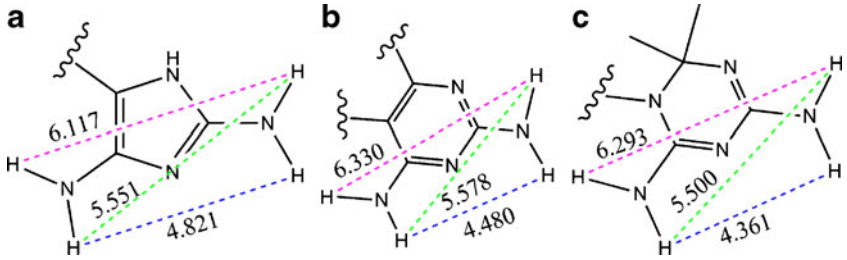


Fig. 4 Distances between the hydrogen atoms of the amino groups of **a** the 1H-imidazole-2,4-diamine scaffold of **4**; **b** the 2,4-diaminopyrimidine moiety of **2**; and **c** the 1,3,5-triazine moiety of **3**. Distances (Å) were obtained from B3LYP/6-31+G* calculations

properties. Moreover, the number of rotatable bonds is <7, which also suggests that the compounds are drug-like [54].

After designing the compounds based on the results of the computational studies discussed above, molecular docking calculations were performed in order to examine their binding modes and affinities in the active sites of the PfDHFR enzymes used in the study. The enzymes used for this study were the wild-type and quadruple mutant PfDHFR enzymes. The Glide program was used to perform the docking calculations. Glide (Gride-based Ligand Docking with Energetics) is one of the most widely used docking programs. It uses a series of hierarchical filters to search for possible locations in the active site region of a receptor [41, 42]. The property of a receptor/active site region is represented by a grid that has different sets of fields that provide progressively more accurate scoring of the ligand pose. It uses a Glide score (Gscore) to predict binding affinity and rank ordering of ligands in database screening. Another scoring function known as Emodel was also used along with Gscore [41, 42]. The docking pose analyses indicated that most of the compounds could form H-bond interactions with the key amino acid residues as expected. The binding modes almost all the compounds were similar to that the experimental binding modes of **3** [5, 7–9]. The aromatic tails of all the compounds occupied the space occupied by the trichloro phenoxy tail of **3** near the opening of the active site. These tails were found to interact hydrophobically with amino acid residues in the hydrophobic region of the active site of the enzyme. Their binding score (Gscore) values are much better than that of **3** for quadruple mutant PfDHFR enzyme, whereas for the wild-type enzyme the scores were found to be comparable to that of **3** (Table 2). Since our objective was to design compounds with equal probabilities to be inhibitors of the wild-type and mutant varieties of these enzymes, only those compounds that were able to dock into the active sites of the wild-type and quadruple mutant PfDHFR enzymes and whose binding conformations super imposed on the experimental conformations of **3** were selected. In most of the compounds, the 2-amino group of the 1H-imidazole-2,4-diamine moiety formed a H-bond interaction with amino acid residue Asp54, whereas the 4-amino group formed two H-bond interactions with the backbone amino acid residues Ile14 and Leu164. Moreover, in some of the compounds the H atom of N^1H was found to orient within a distance <3.00 Å from the second oxygen atom of the carbonyl group of Asp54, suggesting the possibility of forming a bidentate H-bond interaction. In some of the compounds, the 2-amino group of the 1H-imidazole-2,4-diamine moiety was found to form two H-bond interactions with the backbone amino acid residues Ile14 and Leu164, whereas the 4-amino group showed a H-bond interaction with Asp54. In such compounds, the H atom of N^1H the 1H-imidazole-2,4-diamine core is oriented towards Leu164. In all the compounds, the hydrophobic aromatic tails occupy the hydrophobic region near the opening of the active site, and were found to interact via hydrophobic

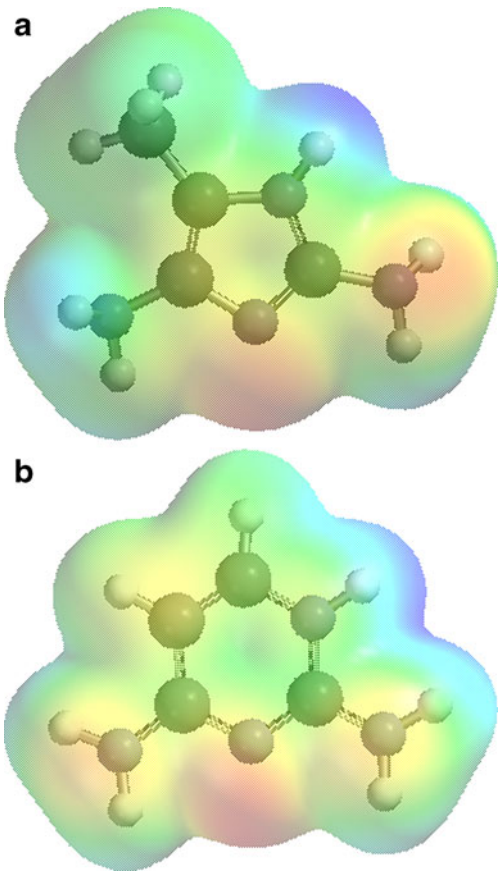
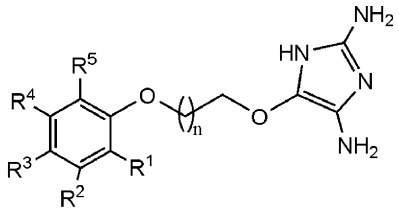
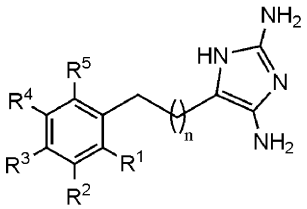


Fig. 5 Molecular electrostatic potential (MESP) surfaces of **a** the 1H-imidazole-2,4-diamine core of **4**, and **b** the N1-protonated 2,4-diaminopyrimidine core of **2**. *Red* Negative potential, *blue* positive potential



6: $R^1 = R^3 = R^4 = Cl$, $R^2 = R^5 = H$, n = 2
7: $R^1 = R^3 = R^4 = OMe$, $R^2 = R^5 = H$, n = 2
8: $R^2 = R^3 = R^4 = Cl$, $R^2 = R^5 = H$, n = 2
9: $R^2 = R^3 = R^4 = OMe$, $R^1 = R^5 = H$, n = 2
10: $R^1 = R^5 = H$, $R^2 = R^3 = R^4 = Cl$, n = 1
11: $R^1 = R^5 = H$, $R^2 = R^3 = R^4 = OMe$, n = 1
12: $R^2 = R^3 = R^4 = Cl$, $R^1 = R^5 = H$, n = 1



13: $R^1 = R^3 = R^4 = Cl$, $R^2 = R^5 = H$, n = 4
14: $R^1 = R^3 = R^4 = OMe$, $R^2 = R^5 = H$, n = 4
15: $R^1 = R^5 = H$, $R^2 = R^3 = R^4 = Cl$, n = 4
16: $R^1 = R^5 = H$, $R^2 = R^3 = R^4 = OMe$, n = 4
17: $R^1 = R^3 = R^4 = Cl$, $R^2 = R^5 = H$, n = 3
18: $R^1 = R^3 = R^4 = OMe$, $R^2 = R^5 = H$, n = 3
19: $R^1 = R^5 = H$, $R^2 = R^3 = R^4 = Cl$, n = 3
20: $R^1 = R^5 = H$, $R^2 = R^3 = R^4 = OMe$, n = 3

Fig. 6 The chemical structures of the designed compounds

interactions with amino acid residues such as Met55, Pro113, Lys46 and Ser111. Substituents such as OMe and Cl on the aromatic tail are also expected to contribute positively to enhancing the hydrophobic interaction of the compounds with the amino acid residues. These amino acid residues are also known to interact with trichloro phenoxy tail of **3** [6, 8, 9, 22, 52]. As demonstrated by the quantum chemical computations, isomer **5** is marginally less stable than isomer **4** (Table 1). This suggested that there could be an easy inter-conversion between these isomers. Thus, molecular docking studies were also carried out on compounds with a 1H-imidazole-2,5-diamine core. The results were similar to those of the compounds with 1H-imidazole-2,4-diamine core (data not shown). The details of the binding poses of the designed ligands (with 1H-imidazole-2,4-diamine core) will be discussed by taking some of the compounds (**6**, **10**, **13** and **17**) as examples. Unless otherwise stated specifically, all discussion is based

Table 2 Molecular weight (MW), number of hydrogen bond donors (HBD), number of hydrogen bond acceptors (HBA), and estimated LogP and Gscore values of the designed compounds in the active sites of quadruple mutant and wild-type *Plasmodium falciparum* dihydrofolate reductase (PfDHFR) enzymes

Compound	MW	No. of HBD	No. HBA	Estimated LogP[d]	Gscore (Emodel)	
					Quadruple mutant	Wild-type
3 (reference)	394.68	2	5	2.92	−8.44[a] (−80.2)	−10.66[a] (−82.4)
6	351.62	3	3	3.10	−10.62[b] (−74.6)	−9.58[a] (−69.7)
7	338.36	3	6	1.06	−9.56[c] (−73.0)	−9.54[a] (−72.3)
8	351.62	3	3	3.10	−9.69[c] (−72.7)	−10.20[a] (−64.6)
9	338.36	3	6	1.06	−8.98[a] (−72.9)	−9.86[a] (−72.3)
10	337.59	3	3	3.04	−11.03[c] (−72.4)	−9.40[a] (−70.2)
11	324.33	3	6	0.99	−9.69[a] (−72.0)	−9.45[a] (−68.4)
12	337.59	3	3	3.04	−10.52[a] (−68.9)	−10.05[a] (−57.3)
13	347.07	3	1	4.97	−10.58[a] (−68.7)	−10.87[a] (−70.2)
14	324.33	3	4	2.93	−9.46[a] (−69.2)	−9.97[a] (−66.0)
15	347.07	3	1	4.97	−9.62[a] (−69.1)	−10.45[a] (−73.3)
16	334.41	3	4	2.93	−9.11[b] (−73.8)	−9.68[a] (−61.4)
17	333.64	3	1	4.51	−10.67[a] (−66.8)	−9.36[b] (−73.3)
18	320.39	3	4	2.47	−9.25[a] (−68.0)	−9.68[b] (−72.3)
19	333.64	3	1	4.51	−9.22[a] (59.4)	−10.98[a] (−67.0)
20	320.39	3	4	2.47	−10.28[a] (−70.1)	−10.21[a] (−66.3)

[a] Compounds showing H-bond interactions with amino acid residues at 14, 54 and 164

[b] Compounds showing H-bond interaction with amino acid residues at 54,14 and 164. In these compounds, there is additional H-bond interaction between Asp54 and N^1H

[c] Compounds showing H-bond interactions with amino acid residues at 14, 54, 164 and 108 in the active sites of PfDHFR enzymes

[d] LogP values were estimated using TOPKAT software implemented in Accelrys Discovery Studio 2.5 [44]

only on the docking results obtained from the active site of the quadruple mutant PfDHFR enzyme.

The docking mode of compound **6** shown in Fig. 7a shows all the key H-bond interactions with amino acid residues Ile14 and Asp54. The observed H-bond length values are 2.230 and 1.805 Å, respectively. One of the oxygen atoms of the linker unit formed an H-bond interaction with the amino groups of Asn108 (2.161 Å). The corresponding interactions of **3** with Ile14 and Asp54 are 1.600 and 2.000 Å, respectively. Moreover, the H atom of N^1H interacts with one of the oxygen atoms of the carbonyl group of Asp54 to form a bidentate H-bond. This type of interaction is common in protonated antifolates, and is believed to increase the binding interactions of ligands with DHFR enzyme. Consistent with that of the trichlorophenyl tail of **3**, the aromatic tail (and substituents on the aromatic tail) of **6** interacts hydrophobically with the amino acid residues near the opening of the active site (e.g., Met55, Ser111 and Pro113).

The binding mode of compound **10** (Fig. 7b) also forms all the required H-bond interactions with the key amino acid residues Ile14, Asp54 and Leu164. The observed H-bond length values are 2.063, 1.969 and 1.961 Å, respectively. These values are also comparable to that of compound **3**. An additional H-bond interaction was observed between one of the oxygen atoms of the linker with Asn108. The aromatic hydrophobic tail and its Cl substituents are also expected to interact hydrophobically with amino acid residues such as Ser111, Pro113 and Met55.

As shown in Fig. 7c, compound **13** forms all the required H-bond interactions with the key amino acid residues Ile14, Asp54 and Leu164. The corresponding H-bond length values are 1.989, 1.879 and 2.273 Å, respectively. As expected (cf. **6**), the H atom of N^1H group formed a bidentate H-bonding interaction with Asp54. Visual inspection also showed that its side chain interacts with amino acids (Met55, Ser111 and Pro113) in the hydrophobic region of the active site.

Figure 7d shows the binding mode of compound **17**. The interactions of this compound show that the compound forms the expected key H-bond interactions with Ile14,

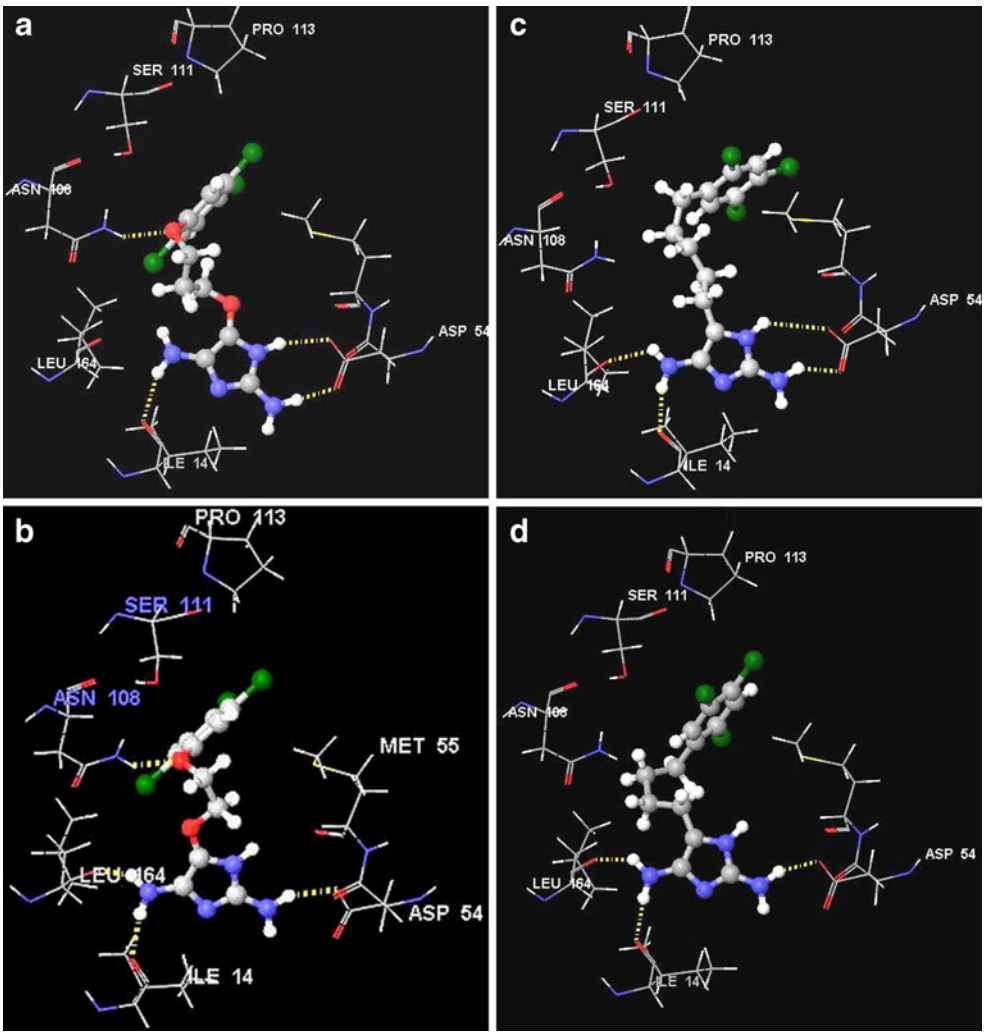


Fig. 7 The binding modes of **6** (**a**), **10** (**b**), **13** (**c**) and **17** (**d**) in the active site quadruple mutant PfDHFR enzyme (1I3K:pdb). For the sake of clarity, only important amino acid residues are shown

Asp54 and Leu164. The corresponding bond length values are 2.029, 2.010 and 2.066 Å, respectively. As with the compounds discussed above, the 1H-imidazole-2,4-diamine moiety of this compound forms strong H-bond interactions with the H-bond acceptor key amino acid residues of the enzyme. Like the compounds discussed above, **17** also interacts with amino acids (Met55, Ser111 and Pro113) in the hydrophobic region of the active site (Fig. 7d). Moreover, no potential steric clash is expected with Asn108.

Drugs (or drug candidates) may fail at a late stage of development or may show unexpected side effects in humans because of poor absorption, distribution, metabolism, elimination and toxicity (ADMET) properties. A significant number of approved and marketed drugs have also been withdrawn due to severe adverse effects [55, 56]. This indicates that in vitro and in vivo tests alone cannot help to make accurate predictions of human drug safety to avoid the marketing of drugs with potential (or unanticipated) adverse effects. This is also a common problem with currently used antimalarial drugs [57, 58]. Therefore, a new paradigm for toxicity testing that combines the strengths of the in vivo, in vitro and in silico approaches is being used to aid prediction of the ADMET profiles of drug candidates in order to reduce attrition rates at the late drug development stage and also to avoid human health risk [59, 60].

One approach to in silico toxicity predictions involves statistical comparison of the characteristics of chemical interest and those compounds with known properties in a database. TOPKAT (Toxicity Prediction by Komputer Assisted Technology) is a widely used system of this type. TOPKAT is a commercially available method used for in silico prediction of toxicities of compounds, and is used by pharmaceutical companies and academic institutions in drug discovery and development programs [61, 62]. It accurately and rapidly assesses the toxicity of chemicals solely from their 2D molecular structure. TOPKAT applies statistically robust, cross-validated quantitative structure–toxicity relationship (QSTR) models for assessing specific toxicological effects [63]. The potential toxicities of our compounds were predicted using this software implemented in Accelrys Discovery Studio 2.5 [44].

Table 3 summarises the results of the toxicity predictions of the designed compounds using five models (Ames mutagenicity, female rat NTP carcinogenicity, male rat NTP carcinogenicity, skin irritation and rat oral LD50). The results indicated that all the compounds (including the reference compound **3**) are non-mutagenic in the Ames mutagenicity prediction model. Experimentally, compound **3** is known to show a negative response in Ames mutagenicity tests [64]. This suggests that the designed compounds will most likely be non-mutagenic in in vivo and in vitro tests. In the NTP carcinogenicity models (female rat NTP carcinogenicity and male rat NTP carcinogenicity) most of the compounds are predicted to be non-carcinogenic. In these models, only compound **7**

Table 3 TOPKAT-predicted properties of the designed compounds

Compound	Ames prediction	Rat female NTP prediction	Rat male NTP prediction	Skin irritancy	Rat oral LD50[a]
6	Non-mutagen	Non-carcinogen	Carcinogen	None	1.00745
7	Non-mutagen	Carcinogen	Carcinogen	None	1.98700
8	Non-mutagen	Non-carcinogen	Non-carcinogen	None	0.70707
9	Non-mutagen	Non-carcinogen	Carcinogen	None	1.77181
10	Non-mutagen	Non-carcinogen	Non-carcinogen	None	1.29214
11	Non-mutagen	Non-carcinogen	Carcinogen	None	1.85202
12	Non-mutagen	Non-carcinogen	Non-carcinogen	None	0.90688
13	Non-mutagen	Non-carcinogen	Non-carcinogen	None	1.22225
14	Non-mutagen	Non-carcinogen	Non-carcinogen	None	1.59095
15	Non-mutagen	Non-carcinogen	Non-carcinogen	None	0.57700
16	Non-mutagen	Non-carcinogen	Non-carcinogen	None	1.32511
17	Non-mutagen	Non-carcinogen	Non-carcinogen	None	1.10082
18	Non-mutagen	Non-carcinogen	Non-carcinogen	None	1.43050
19	Non-mutagen	Non-carcinogen	Non-carcinogen	None	0.51968
20	Non-mutagen	Non-carcinogen	Non-carcinogen	None	1.19147
3 (ref)	Non-mutagen[b]	Non-carcinogen	Non-carcinogen	Mild	0.30362[c]

[a] g/kg body weight

[b] Reported to show negative test [64]

[c] Reported rat oral LD50 of compound **3** is 1.980 g/kg body weight [65, 66]

was predicted to be carcinogenic whereas compounds **6**, **9**, **11** and **13** were predicted to be carcinogenic in male rat NTP carcinogenicity model (Table 3). Moreover, all the compounds were predicted to be non-irritant in the skin irritancy model. The results also show compound **3** as mild skin irritant. The rat oral LD50 values of all the compounds were predicted to be less than 2 g/kg body weight (Table 3).

Conclusions

The main objective of the CAMD approach is to expedite and facilitate hit identification, hit-to-lead selection and lead optimization by improving pharmaceutical and ADMET properties with reduced time and expense. In our study, we employed this approach to design 1H-imidazole-2,4-diamine derivatives as potential inhibitors of wild-type and quadruple mutant PfDHFR enzymes. The compounds have flexible side chains that could avoid unfavourable steric clashes with mutated amino acid residue (e.g., Asn108) in the active site of mutant varieties of PfDHFR enzymes. The 1H-imidazole-2,4-diamine core of the compounds could form optimal H-bond interactions with the enzyme, similar to those of the 2,4-diaminopyrimidine and 1,3,5-triazine cores of known antifolates. The results of molecular docking studies also showed that the compounds have similar binding modes with that of the experimental ligand (**3**) in the active site regions of both wild-type and quadruple mutant PfDHFR enzyme. The docking scores are also comparable to those of **3**. Moreover, the results suggest that the compounds could be effective inhibitors against the other mutant varieties of the PfDHFR enzymes harbouring double and triple mutations. The in silico toxicity predictions also indicate that the designed compounds are non-toxic. Our literature survey indicated no reports on antimalarial activities of 1H-imidazole-2,4-diamine derivatives. All these facts suggest that these compounds are suitable candidates for further pharmacological studies to explore their antimalarial activities. During the design of the compounds, their synthetic accessibility was also considered. Since reagents with a 1H-imidazole-2,4-diamine scaffold are available commercially, the designed compounds are synthetically accessible. Thus, we encourage research teams working in the area of antimalarial drug discovery to take up the synthesis and activity test steps so that compounds with high activity against the wild-type and mutant PfDHFR enzymes can be identified to combat malaria.

Acknowledgement L.A. thanks The Government of Federal Democratic Republic of Ethiopia for financial support.

References

1. Schenell JR, Dyson HJ, Wright PE (2004) Structure, dynamics, and catalytic function of dihydrofolate reductase. Annu Rev Biophys Biomol Struct 33:119–140
2. Nzila A (2006) Inhibitors of de novo folate enzymes in *P. falciparum*. Drug Discov Today 11:939–944
3. Anderson AC, Wright DL (2005) Targeting DHFR in parasitic protozoa. Drug Discov Today 10:121–128
4. Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI (2005) The global distribution of clinical episodes of *P. falciparum* malaria. Nature 434:214–217
5. Lemcke T, Christensen IT, Jorgensen FS (1999) Towards understanding of drug resistance in malaria: three-dimensional structure of *P. falciparum* DHFR by homology building. Bioorg Med Chem 7:1003–1011
6. Rastelli G, Sirawaraporn W, Sompornpisut P, Vilaivan T, Kamchonwongpaisan S, Quarrell R, Lowe G, Thebtaranonth Y, Yuthavong Y (2000) Interaction of pyrimethamine, cycloguanil, WR99210 and their analogues with *P. falciparum* dihydrofolate reductase: structural basis of antifolate. Bioorg Med Chem 8:1117–1128
7. Delfino RT, Santos-Filho OA, Figueroa-Villar JD (2002) Molecular modeling of wild-type and antifolate resistant mutant *P. falciparum* DHFR. Biophys Chem 98:287–300
8. Santos-Filho OA, de Alencastrob RD, Figueroa-Villar JD (2001) Homology modeling of wild-type and pyrimethamine/cycloguanil-cross-resistant mutant type *P. falciparum* DHFR: a model for antimalarial chemotherapy resistance. Biophys Chem 91:305–317
9. Yuvaniyama J, Chitnumsub P, Kamchonwongpaisan S, Vanichatanankul J, Sirawaraporn W, Taylor P, Walkinshaw MD, Yuthavong Y (2003) Insights into antifolate resistance from malarial DHFR-TS structures. Nat Struct Biol 10:357–365
10. Sirawaraporn W, Sathitkul T, Sirawaraporn R, Yuthavong Y, Santi DV (1997) Antifolate-resistant mutants of *P. falciparum* dihydrofolate reductase. Proc Natl Acad Sci USA 94:1124–1129
11. Warhurst DV (1998) Antimalarial drug discovery: development of inhibitors of dihydrofolate reductase active in drug resistance. Drug Discov Today 3:538–546
12. Legesse A, Bharatam PV (2009) 3D-QSAR analysis of cycloguanil derivatives as inhibitors of Dihydrofolate reductase enzyme from Cycloguanil-resistant strain (T9/94) of *P. falciparum*: CoMFA and CoMSIA studies. J Mol Graph Model 28:357–367
13. Hecht D, Cheung M, Fogel GB (2008) QSAR using evolved neural networks for the inhibition of mutant *Pf*DHFR by pyrimethamine derivatives. BioSystems 92:10–15
14. Maitarad P, Saparpakorn P, Hannongbua S, Kamchonwongpaisan S, Tarnchompoo B, Yuthavong Y (2009) Particular interaction between pyrimethamine derivatives and quadruple mutant type dihydrofolate reductase of *P. falciparum*: CoMFA and quantum chemical calculations studies. J Enzyme Inhib Med Chem 24:471–479
15. Maitarad P, Kamchonwongpaisan S, Vanichtanankul J, Vilaivan T, Yuthavong Y, Hannongbua S (2009) Interactions between cycloguanil derivatives and wild type and resistance-associated mutant *P. falciparum* dihydrofolate reductases. J Comput Aided Mol Des 23:241–252
16. Delfino RT, Santos-Filho OA, Figuar-Villar JD (2002) Type 2 antifolates in the chemotherapy of falciparum malaria. J Braz Chem Soc 13:727–741
17. Legesse A, Bharatam PV (2008) Modelling and informatics in the analysis of *P. falciparum* DHFR enzyme inhibitors. Curr Med Chem 15:1552–1569
18. McKie JH, Douglas KT, Chan C, Roser SA, Yates R, Read M, Hyde JE, Dascombe MJ, Yuthavong Y, Sirawaraporn W (1998)

Rational drug design approach for the overcoming drug resistance: application to pyrimethamine resistance in malaria. J Med Chem 41:1367–1370
19. Sardarian A, Douglas KT, Read M, Sims PF, Hyde JE, Chitnumsub P, Sirawaraporn R, Sirawaraporn W (2003) Pyrimethamine analogs as strong inhibitors of double and quadruple mutants of dihydrofolate reductase in human malaria. J Biol Chem 1:960–964
20. Kamchonwongpaisan S, Quarrell R, Charoensetakul N, Ponsinet R, Vilaivan T, Vanichtanankul J, Tarnchompoo B, Sirawaraporn W, Lowe G, Yuthavong Y (2004) Inhibitors of multiple mutants of *P. falciparum* dihydrofolate reductase and their antimalarial activities. J Med Chem 47:673–680
21. Yuthavong Y, Vilainvan T, Chareonsethakul N, Kamchonwongpaisan S, Sirawaraporn W, Quarrell R, Lowe G (2000) Development of a lead inhibitor for the A16V + S108T mutant of dihydrofolate reductase from the cycloguanil-resistant strain (T9/94) of *P. falciparum*. J Med Chem 43:2738–2744
22. Sichaiwat C, Intaraudom C, Kamchonwongpaisan S, Vanichtanankul J, Thebtaranonth Y, Yuthavong Y (2004) Target guided synthesis of 5-benzyl-2, 4-diamonopyrimidines: their antimalarial activities and binding affinities to wild type and mutant dihydrofolate reductases from P. falciparum. J Med Chem 47:345–354
23. Hunt SY, Detering C, Varani G, Jacobus DP, Schiehser GA, Shieh HM, Nevchas I, Terpinski J, Sibley CH (2005) Identification of the optimal third generation antifolate against *P. falciparum* and *P. vivax*. Mol Biochem Parasitol 144:198–205
24. Japrung D, Leartsakulpanich U, Chusacultanachai S, Yuthavong Y (2007) Conflicting requirements of *P. falciparum* dihydrofolate reductase mutations conferring resistance to pyrimethamine-WR99210 combination. Antimicrob Agents Chemother 51:4356–4360
25. Legesse A, Patel DS, Bharatam PV (2010) Shape- and chemical feature-based virtual screening: identification of leads as potential inhibitors of *Pf*DHFR enzyme. Chem Biol Drug Des 75:115–126
26. Bhattacharjee AK, Nichols DA, Gerena L, Roncal N, Gutteridge CE (2007) An *in silico* 3D pharmacophore model of chalcones useful in the design of novel antimalarial agents. Med Chem 3:317–326
27. França TC, Wilter A, Ramalho TC, Pascutti PG, Figueroa-Villar JD (2006) Molecular dynamics of the interaction of *Plasmodium falciparum* and human serine hydroxymethyltransferase with 5-formyl-6-hydrofolic acid analogues: design of new potential antimalarials. J Braz Chem Soc 17:1383–1392
28. Dasgupta T, Chitnumsub P, Kamchonwongpaisan S, Maneeruttanarungroj C, Nichols SE, Lyons TM, Tirado-Rives J, Jorgensen WL, Yuthavong Y, Anderson KS (2009) Exploiting structural analysis, in silico screening, and serendipity to identify novel inhibitors of drug-resistant falciparum malaria. ACS Chem Biol 4:29–40
29. Desai PV, Patny A, Gut J, Rosenthal PJ, Tekwani B, Srivastava A, Avery M (2006) Identification of novel parasitic cysteine protease inhibitors by use of virtual screening. 2. The available chemical directory. J Med Chem 49:1576–1584
30. Hehre WJ, Radom L, Schleyer PV, Pople JA (1995) Ab initio molecular orbital theory. Wiley, New York
31. Forseman JB, Frisch AE (1998) Exploring chemistry with electronic structure methods. Gaussian, Pittsburg
32. Par RG, Yang W (1989) Density functional theory of atoms and molecules. Oxford University Press, New York
33. Bartolotti LJ, Fluchick K (1996) In: Lipkowitz KB (ed) Reviews in computational chemistry. VCH, New York
34. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery JJA, Vreven T, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Gonzalez C, Pople JA (2004) Gaussian 03, Revision C.02. Gaussian Inc, Wallingford. http://www.gaussian.com
35. Becke AD (1993) Density functional thermochemistry. III. The role of exact exchange. J Chem Phys 98:5648–5652
36. Lee C, Yang W, Parr RG (1988) Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Phys Rev B37:785–789
37. Perdew JP, Wang Y (1992) Accurate and simple analytic representation of the electron-gas correlation energy. Phys Rev B45:13244–13249
38. Scott AP, Radom L (1996) Harmonic vibrational frequencies: an evaluation of Hartree-Fock, Møller-Plesset, Quadratic configuration interaction, density functional theory, and semiempirical scale factors. J Chem Phys 100:16502–16513
39. Barone V, Cossi M (1998) Quantum calculation of molecular energies and energy gradients in solution by a conductor solvent model. J Phys Chem 102:1995–2001
40. Hehre WJ (2001) A guide to molecular mechanics and quantum chemical calculations. Wavefunction, Irvine
41. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, Repasky MP, Knoll EH, Shelley M, Perry JK, Shaw DE, Francis P, Shenkin PS (2004) Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. J Med Chem 47:1739–1749
42. Halgren TA, Murphy RB, Friesner RA, Beard HS, Frye LL, Pollard WT, Banks JL (2004) Glide: a new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. J Med Chem 47:1750–1759
43. SYBYL 7.1 (2005) Tripos, St Louis, MO
44. http://accelrys.com/products/discovery-studio/toxicology/index.html (Accessed on 2 January, 2010)
45. Taft CA, da Silva VB, da Silva CHT (2008) Current topics in computer-aided drug design. J Pharm Sci 97:1089–1098
46. Song CM, Lim SJ, Tong JC (2009) Recent advances in computer-aided drug design. Briefings Bioinform 10:579–591
47. Kapetanovic IM (2008) Computer-aided drug discovery and development (CADDD): in silico-chemico-biological approach. Chem Biol Interact 171:165–176
48. Patel DS, Bharatam PV (2009) Novel $^{+}N(\leftarrow L)_2$ species with two lone pairs on nitrogen: systems isoelectronic to carbodicarbenes. Chem Commun 2009:1064–1066
49. Bharatam PV, Patel DS, Iqbal P (2005) Pharmacophoric features of biguanidienes: an electronic structure study. J Med Chem 48:7615–7622
50. Bharatam PV, Sundriyal S (2006) Molecular electrostatic potentials in the design of dendrimers for the delivery of glitazones. J Nanosci Nanotechnol 6:3277–3282
51. Roy DK, Balanarayan P, Gadre SR (2009) Signatures of molecular recognition from the topography of electrostatic potential. J Chem Sci 121:815–821
52. Rastelli G, Pacchioni S, Sirawaraporn W, Sirawaraporn R, Parenti MD, Ferrari AM (2003) Docking and database screening reveal new classes of *P. falciparum* dihydrofolate reductase inhibitors. J Med Chem 46:2834–2845

53. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 23:3–25
54. Vistoli G, Pedretti A, Testa B (2008) Assessing drug likeness—what we are missing? Drug Discov Today 13:285–294
55. Li AP (2004) Accurate prediction of human drug toxicity: a major challenge in drug development. Chem Biol Interact 150:3–7
56. Drug withdrawals. http://www.ganfyd.org. (Accessed 25 December 2009)
57. Chattopadhyay R, Mahajan B, Kumar S (2007) Assessment of safety of the major antimalarial drugs. Expert Opin Drug Safety 6:505–521
58. Alkadi HO (2007) Antimalarial drug toxicity. Chemotherapy 53:385–391
59. Gifford E, van de Waterbeemd H (2003) ADMET in silico modeling: towards prediction paradasie? Nat Rev Drug Discov 2:192–204
60. Merlot C (2010) Computational toxicology: a tool for early safety evaluation. Drug Discov Today 15:16–22
61. Prival MJ (2001) Evaluation of the TOPKAT system for predicting the carcinogenicity of chemicals. Environ Mol Mutagen 37:55–69
62. Richard AM (1998) Commercial toxicology prediction systems: a regulatory perspective. Toxicol Lett 102:611–616
63. TOPKAT Version 6.2. User Guide (2004) Accelrys, San Diego
64. Genther CS, Schoeny RS, Loper JC, Smith CC (1977) Mutagenic studies of folic acid antagonists. Antimicrob Agents Chemother 12:84–92
65. Mui EJ, Schiehser GA, Milhous WK, Hsu H, Roberts CW, Kirisits M, Muench S, Rice D, Dubey JP, Fowble JW, Rathod PK, Queener SF, Liu SR, Jacobus DP, McLeod R (2008) Novel Triazine JPC-2067-B inhibits *Toxoplasma gondii* in vitro and in vivo. PLoS Negl Trop Dis 2:e190. doi:10.1371/journal.pntd.0000190
66. Canfield CJ, Milhous WK, Ager AL, Rossan RN, Sweeney TR, Lewis NJ, Jacobus DP (1993) PS-15: a potent, orally active antimalarial from a new class of folic acid antagonists. Am J Trop Med Hyg 49:121–126