Received Date : 26-Jun-2014

Revised Date : 21-Nov-2014

Accepted Date : 24-Nov-2014

Article type : Research Article

Synthesis, docking, *in vitro* and *in vivo* antimalarial activity of hybrid 4-aminoquinoline-1,3,5-triazine derivatives against wild and mutant malaria parasites

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Running head title

Bhat *et al.*: Synthesis and antimalarial activity of 4-aminoquinoline and 1,3,5-triazine conjugates.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/cbdd.12490

Abstract

A new series of hybrid 4-aminoquinoline 1,3,5-triazine derivatives was synthesized by a four-step reaction. Target compounds were screened for *in vitro* antimalarial activity against chloroquine-sensitive (3D-7) and chloroquine-resistant (RKL-2) strains of *P. falciparum.* Compounds exhibited, by and large, good antimalarial activity against the resistant strain while two of them, i.e., **8g** and **8a** displayed higher activity against both the strains of *P. falciparum.*Additionally, docking study was performed on both wild (1J3I.pdb) and qradruple mutant (N51I, C59R, S108 N, I164L, 3QG2.pdb) type *pf*-DHFR-TS to highlight the structural features of hybrid molecules.

Key word: Aminoquinoline, 1,3,5-triazine, antimalarial, docking.

Introduction

According to the recent report of the World Health Organization (WHO), an estimated 3.3 billion people were at risk of malaria and approximately 80% malaria cases were observed in African region [1]. Accordingly, discovery of novel antimalarial compounds endowed high therapeutic efficiency along with antimalarial potential against resistance strains is always a topic of interest [2,3]. In this regard, 1,3,5-triazine derivatives received considerable attention, for instance, proguanil, a prodrug of cycloguanil belongs to 1,3,5-triazine containing antimalarial drug act via arresting of *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase (*pf*-DHFR-TS), an enzyme required in biochemical processes that are vital for parasite growth [4, 5]. Impressed by the antimalarial efficacy of proguanil, various derivatives of 1,3,5-triazine were also synthesized in search of novel drug entity, e.g., cycloguanil, chlorcycloguanil, clociguanil, WR99210 [6].

In our previous studies, we have designed several analogues of 1,3,5-triazine with 4-aminoquinoline as effective antimalarial agents [7]. During the course of study it has been observed that the structure–activity relationship (SAR) could be exemplified on the nature of linker between 4-aminoquinoline and 1,3,5-triazine pharmacophore and variety of fragment attached to the other two wings of 1,3,5-triazine moties [8,9]. The SAR of antimalarial agents clearly suggests the use of basic compounds and its strong influence on the antimalarial activity [10]. The structural input drawn from our previous studies has allowed us to devise new series of compounds where two pharmacophores *i.e.*, 4-aminoquinoline and 1,3,5-triazine have been tethered via 2-(piperazin-1-yl) ethylamine basic linker. These targeted molecules were subsequently screened against chloroquine sensitive (3D-7) and chloroquine resistant (RKL-2) strains of *Plasmodium falciparum.* Furthermore, molecular docking study was also performed to get insight of essential key structural requirements for antimalarial activity.

Methods and Materials

The solvents and reagents of analytical grade were used without further purification. Melting points were determined on a Veego MPI melting point apparatus and FT-IR (2.0 cm^{-1}, flat, smooth, abex) were recorded on Perkin Elmer RX-I Spectrophotometer. ^{1}H-NMR spectra were recorded on Bruker Avance II 400 NMR and ^{13}C-NMR spectra on Bruker Avance II 100 NMR spectrometer in DMSO-d_6 using TMS as internal standard. Mass spectra were obtained on VG-AUTOSPEC spectrometer equipped with electrospray ionization (ESI) source. Elemental analysis was carried out on Vario EL-III CHNOS elementor analyzer.

The desired compounds **3**, **6**, **7a-i** and **8a-i** were synthesised using the protocols as outlined in Scheme 1. Initially the synthesis of compound **3** was achieved by the nucleophilic

substitution of N–aminoethyl piperazine (**2**) at the C-4 chloro of 4,7-dichloroquinoline (**1**). The mono-substituted 1,3,5-triazines, such as 4,6-dichloro-N-(4-nitrophenyl)-1,3,5-triazin-2-amine (**6**) was accomplished by the nucleophilic substitution of the Cl atom of the 2,4,6-trichloro-1,3,5-triazine (**4**) with *p*-nitro aniline (**5**) in the presence of saturated solution of $NaHCO_3$. Further, the synthesis of di-substituted 1,3,5-triazines **7a-i** was accomplished by the nucleophilic substitution at one of the Cl atom of compound **6** with different primary and secondary amines (**a-i**), as presented in scheme 1. The final targeted compounds (**8a-i)** were realised upon treating **7a-i** with compound **3**.

The synthesis of intermediate compounds **3**, **6**, **7a-i** was performed according earlier reported procedure [9,11].

General procedure for synthesis of compounds **8a-i**

A solution of respective di-substituted 1,3,5-triazine compounds **7a-i** (0.01eq.) and compound **3** (0.01 eq.) and K_2CO_3 (0.01 eq.) in 1,4-dioxane was refluxed for 8-9h. The completion of reaction was monitored by TLC using ethanol:acetone (1:1) as mobile phase. The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was re-crystallised from ethanol to afford the desired products **8a-i**.

The spectral and analytical data of final compounds as well as the intermediates were given in Supplementary information

Antimalarial Activity

Preparation of parasites

The chloroquine sensitive 3D-7 and chloroquine resistant RKL-2 strains (Rourkela, Orissa, India) of *P. falciparum* were routinely maintained in stock cultures in medium RPMI-1640 supplemented with 25mmol HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. The asynchronous parasites were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, the initial ring stage parasitaemia of 1.0 % at 3.0 % haematocrit in a total volume of 200 µL of medium RPMI-1640 was uniformly maintained.

In-vitro antimalarial assay

The *in-vitro* antimalarial assay was carried out according to microassay process described by Reickmann and co-workers using 96 well-microtitre plates [12] with minor modifications. A stock solution of 5mg/mL of each test compound was prepared in DMSO and subsequent dilutions were prepared with culture medium. The test compounds in 20 µL volume at two concentrations, i.e., 5 µg/mL and 50 µg/mL in triplicate wells were incubated with parasitized cell preparation at 37 ºC in CO_2 incubator set at 37 ^{o}C and 5% CO_2 level . After 40h of incubation, the blood smears were prepared from each well and stained with Giemsa stain. The level of parasitemia in terms of percentage dead rings along with trophozoites was determined by counting a total of 100 asexual parasites (both live and dead) in each smear microscopically using chloroquine and proguanil as reference drugs.

In vivo antimalarial assay

The *in vivo* drug response was evaluated in swiss albino mice infected with *P. berghei,* which is innately sensitive to chloroquine [13]. The mice (20±5 g) was inoculated with $1x10^7$ parasitized RBC on day 0 and treatment was administered to a group of five mice from day 0-4, once daily. The aqueous suspension of compounds were prepared with 2.5µl of 30% ethanol and diluted with Milli Q grade water. Initially, the efficacy of test compounds was evaluated at 5 mg/kg/day and required daily dose was administered in 0.2 ml volume *via* intraperitoneal route. Parasitaemia levels were recorded from thin blood smears between day 4. The mean value determined for a group of 5 mice was used to calculate the percent suppression of parasitaemia with respect to the untreated control group. Mice treated with chloroquine served as reference control.

Molecular docking studies

The 3D X-ray crystal structure of wild type (1J3I.pdb) and qradruple mutant (N51I, C59R, S108 N, I164L, 3QG2.pdb) *pf*-DHFR-TS were used as starting model for this study. The protein was prepared, docked and its interaction with different ligands was studied using Discovery Studio 2.5 (Accelrys Software Inc., San Diego; http://www.accelrys.com).

Preparation of receptor

The target wild and qradruple mutant *pf*-DHFR proteins without co-crytallised ligands were taken and the bond order corrected. The hydrogen atoms were added and their positions optimized using the all-atom CHARMm (version c32b1) forcefield with Adopted Basis set Newton Raphson (ABNR) minimization algorithm until the root mean square (r.m.s) gradient for potential energy was less than 0.05 kcal/mol/Å [14,15]. Using the 'Binding Site' tool panel available in DS 2.5, the minimized protein structure was defined as receptor, the binding site

was defined as volume occupied by the ligand in the receptor, and an input site sphere was defined over the binding site with a radius of 5 Å. The center of the sphere was taken to be the center of the binding site, and side chains of the residues in the binding site within the radius of the sphere were assumed to be flexible during refinement of postdocking poses. The receptor having defined binding site was used for the docking studies.

Ligand setup

Using the built-and-edit module of DS 2.5, various ligands were built, all-atom CHARMm forcefield parameterization assigned and then minimized using the ABNR method. A conformational search of the ligand was carried out using a stimulated annealing molecular dynamics (MD) approach. The ligand was heated to a temperature of 700 K and then annealed to 200 K. Thirty such cycles were carried out. The transformation obtained at the end of each cycle was further subjected to local energy minimization using the ABNR method. The 30 energy-minimized structures were then superimposed and the lowest energy conformation occurring in the major cluster was taken to be the most probable conformation.

Docking

Docking, a significant computational method, was used to predict the binding of the ligand to the receptor binding site by varying position and conformation of the ligand keeping the receptor rigid. LigandFit [15] protocol of DS 2.5 was used for the docking of ligands with wild and quadruple mutant *pf*-DHFR proteins [16]. The LigandFit docking algorithm combines a shape comparison filter with a Monte Carlo conformational search to generate docked poses consistent with the binding site shape. These initial poses are further refined by rigid body minimization of the ligand with respect to the grid based calculated interaction energy using the Dreiding forcefield [17]. The receptor protein conformation was kept fixed

during docking, and the docked poses were further minimized using all-atom CHARMm (version c32b1) forcefield and smart minimization method (steepest descent followed by conjugate gradient) until r.m.s gradient for potential energy was less than 0.05 kcal/mol/Å. The atoms of ligand and the side chains of the residues of the receptor within 5 Å from the center of the binding site were kept flexible during minimization.

Results and discussion

The hybrid molecules were synthesized following the standard protocols as discussed previously. While, the structure of final derivatives were ascertained on the basis various spectral analysis *viz.*, FT-IR, ^{1}H-NMR,^{13}C-NMR and Mass. The spectroscopic data are in good arrangement with the proposed structures.

Antimalarial activity and structure-activity relationship

In vitro antimalarial screening data against chloroquine-sensitive (3D-7) and chloroquine resistant (RKL-2) strains of *P. falciparum* at 5 µg/mL and 50µg/mL doses are provided in table 1. Hybrid compounds **8a-i** killed parasitemia ranging from 8.0-36.0 % at 50µg/mL and 5.0-20.5% at 5 µg/mL in the case of 3D-7 and 22-45.3% at 50µg/mL and 9.6-22.3% at 5 µg/mL in the case of RKL-2 strains, indicating marginally better activity against the resistant strain. The compound **8g** bearing aliphatic analogs such as semicarbazide containing 1,3,5-triazine moeity was found to exhibit highest activity against both the strains at 50µg/mL concentration. However, compounds **8a-c** synthesized by incorporation of aryl groups on 1,3,5-triazine nucleus exhibited moderate to low activity against both the strains. Compound **8a** substituted with morpholine on 1,3,5-triazine registered 25.5 % parasitemia kill against chloroquine-sensitive and 38.3 % parasitemia kill against chloroquine resistant strain at 50 µg/mL. Furthermore, compound **8b** showed 16 % parasitemia kill against chloroquine-

sensitive and 36.6 % parasitemia kill against chloroquine resistant strain at 50 µg/mL. The compound **8c** substituted with phenol on 1,3,5-triazine showed 8% parasitemia kill against chloroquine–sensitive and 34.6% parasitemia kill against chloroquine resistant strain. Compounds **8d** and **8h** with single methyl group at *para* and *ortho* position of the phenyl ring substituted on 1,3,5-triazine base, respectively, registered a remarkable 13.5 % and 26.5% parasitemia kill against chloroquine-sensitive and 36.6 % and 22% parasitemia kill against chloroquine-resistant strain at 50 µg/mL. The introduction of thiosemicarbazide on the 1,3,5-triazine base resulting in compound **8e** increased the antimalarial activity against both the stains with parasitemia kill of 29 % and 28 % at 50 µg/mL. Among the compounds having thiourea on 1,3,5-triazine moeity, compound **8f** showed 18 % parasitemia kill against chloroquine-sensitive and 40.6% parasitemia kill against chloroquine-resistant strain. The hybrid derivative **8i** with amine group substituted on 1,3,5-triazine ring, recorded 14 % parasitemia kill against chloroquine-sensitive and 34.6 % parasitemia kill against chloroquine-resistant strain at 50 µg/mL.

Among the synthesized derivatives, **8g** were identified as most potent molecule and thus it subsequently evaluated for *in vivo* antimalarial efficacy against chloroquine sensitive rodent malaria parasite (*P. berghei)* in swiss albino mice model using Peters' 4-day suppressive test using chloroquine as reference drug at the dosage of 5 mg/kg body weight. As depicted in table 2, compound **8g,** bearing semicarbazide demonstrate good suppression of parasitaemia (64.5%) with mean survival time 21.3$\pm$4.0 days in comparison to the mean survival time of 22.1 ± 9.2 days obtained with the reference drug chloroquine at 5 mg/kg body weight. Results revealed that introducing more basic semicarbazide moiety (**8g**) resulted in the highest supression of parasitaemia and the highest mean survival time for treated mice.

The structure-activity relationship (SAR) studies, emerged from the screening data showed in Figure 1, indicated that the aliphatic substituent at the 1,3,5-triazine ring such as thiosemicarbazide (**8e)**, thiourea (**8f)**, semicarbazide (**8g)** and amine (**8i)** played a vital role in the activity. While incorporation at *p*-OH (**8c)**, *p*-CH_3 (**8h)**, *p*-OCH_3 (**8b)** and *o*-CH_3 (**8d)** aromatic ring substituted on 1,3,5-triazine ring reduced the antimalarial activity, the introduction of basic moieties, like thiosemicarbazide (**8e**), semicarbazide (**8g**), thiourea (**8f**), mopholine (**8a**), and ammonia (**8i)** at 1,3,5-triazine ring enhanced the activity suggesting that the basic moiety at 1,3,5-triazine was important for antimalarial activity. Our studies indicated that compounds **8b** and **8c** with (*p*-OCH_3 and *p*-OH) substituents on phenyl ring at 1,3,5-triazine ring showed mild to moderate activity against both the strains of *P. falciparum.* Furthermore, methyl group substituted at *ortho* and *para* position of aryl ring (**8d** and **8h)** also showed mild to moderate activity.

Molecular Docking Study

In the given docking protocol, we had carried out molecular dynamics simulations, which calculated the time dependent behaviour of a molecular system and provided detailed information on the fluctuations and conformational changes of proteins. These methods are now routinely used to investigate the structure, dynamics and thermodynamics of biological molecules and their complexes. The CHARMM force field was also applied to the designed molecules, which added the deficient hydrogen atoms in the molecules, minimized energy to stable conformation and analysed the structure for efficient binding with protein. The program can read or model structures, minimize the energy by first- or second-derivative techniques, perform a normal mode or molecular dynamics simulation, and analyse the structural, equilibrium, and dynamic properties as determined in these calculations [18].

The docking studies of all compounds **8a-i** were performed into the binding pocket of both wild type (1j3i.pdb) and qradruple mutant (N51I, C59R, S108 N, I164L, 3QG2.pdb) *Pf*-DHFR-TS. The docking results and docked conformations of ligands in the active site are illustrated in Table 3, while the docking interaction of compound **8g** being the most active one, showed in Figure 2 and Figure 3. In this study, we had found that the molecules formed stable interactions with wild and mutant *Pf*-DHFR-TS enzyme by bringing change in the conformation of the protein.

In a pioneering study by Yuvanyama *et al*., it has been found that the carboxylate oxygen atoms of Asp54 were involved in hydrogen bond formation with N3 and N4-amino nitrogen atoms of WR99210 (a 1,3,5-triazine containing pre-clinical molecule as *Pf*-DHFR-TS inhibitor) or with N1 and N2 amino nitrogen atoms of Pyr, as expected for this class of inhibitors [19]. Similarly, our compound showed the formation of hydrogen bond with the carboxylate oxygen atoms of Asp54 through the involvement of NH groups of 1,3,5-triazine phenyl and semicarbazide in wild-type. Other non-bonded π –π interactions between 1,3,5-triazine phenyl and 1,3,5-triazine ring with Phe58 were also observed. The π - + interactions between 1,3,5-triazine phenyl (NO_2 group) with Phe58 and Leu40 in wild and mutant *Pf*-DHFR-TS were also observed. In *in-vitro* antimalarial activity results showed that the compound **8g** showed highest antimalarial activity against wild-type strain of *P. falciparum,* which was confirmed by the interactions with Ser111. Further, in mutant *Pf*-DHFR-TS showed no favorable interactions with critical amino acid residues *viz.* Ala16, Cys50, Asn51, Cys59 and Ser108. Most of the derivatives bind in more or less similar manner with substituted phenyl ring and triazine nucleus occupying the interior of the deep cleft of the active site by the formation of hydrogen bonds while the quinoline moiety formed π-π, π-+, π-σ interactions with different amino acid residues of the receptor molecule.

After molecular docking analysis of target compounds, it is imperative to discern between sound and bad docking solution, thus scoring function is deemed as a worthy option for it. These functions are traditionally either physics-based or knowledge based and differ mainly in the derivation of the mathematical models. The major implication of the scoring function is rest with their power to calculate the intensity of the non-covalent interaction, called as binding affinity, and other parameters between ligand and receptor after they have been bobbed. So, to better see the molecular interaction and their correlation with biological action, two molecules viz., highly active (**8g**) and non-active (**8c**) compounds were rigorously analysed through various scoring parameters. The outcomes were presented in table 4.

It has been found that compound **8g** being as highly active in bioassay showed prominent values for PLP1 and PLP2 than its non-active counterpart (**8c**). Piecewise Linear Potential (PLP) is a quick and simple docking function that has been presented to elucidate the protein ligand binding affinities in an effective manner. The PLP scores are measured in arbitrary units and higher PLP scores indicate stronger receptor-ligand binding. On comparing the PLP scores, compound **8g** was found much active than **8c** against quardruple mutant form which is found in agreement with anti-malarial activity. In the case of the wild type model, compound **8g** showed lesser affinity than mutant model, but significantly higher than non active compound **8c**.

The PMF scoring functions were developed on the basis of statistical analysis of the 3D structures of protein-ligand complexes. These were found to correlate well with protein-ligand interaction. The scores are computed by summing pairwise interaction terms over all interatomic pairs of the receptor-ligand complex. Compound **8g** was again turned out as

efficient legend (144.52) by exhibiting higher PMF score than **8c** (121.82) against the quadruple mutant protein model. Further, the same pattern is also revealed on comparing the PMF values against wild-type. These results are found in better correlation with *in-vitro* and *in-vivo* anti-malarial activity.

In order to better understand the ligand-protein interaction, molecules (**8g** and **8c**) were further analysed on the basis dock score. The most active molecule **8g** presented significantly higher values against quardruple mutant than compound **8c**. While, the same correlation held true for wild type protein model, i.e., **8g** is found more active than **8c**. It was understood that, compound **8g** was appreciably more active than **8c** for both the protein model, showing higher correlation with *in-vitro* and *in- vivo* antimalarial activity.

Conclusion:

In conclusion, a new series of hybrid 4-aminoquinoline 1,3,5-triazine was designed and synthesized. In the present series, compound **8g** showed good *in vitro* antimalarial activity against both chloroquine sensitive as well as resistant strains of *P. Falciparum* and also excellent *in vivo* antimalarial activity against *P. Berghei.* Activity results indicated that compound **8g** may be utilized as lead molecules for further chemical modifications to improve their therapeutic potential as antimalarial agents.

Acknowledgement

The financial support from the Department of Science and Technology (DST), New Delhi, India is gratefully acknowledged (Grant no. SR/SO/HS-125/2010). Authors are also thankful to S.A.I.F., Punjab University, Chandigarh, India for providing spectroscopic data.

Conflict of interest: Authors declare no conflict of interest.

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Figures and Schemes captions

Scheme 1 : Reagents and conditions: R-H (a-i) various amines, (i) Reflux 1hr at 80^{o}c followed by 6-8 h at 120-130^{o}C (ii) Acetone, 0-5 ^{o}C 3h, $NaHCO_3$ (iii) Acetone, 40-45 ^{o}C, 5 h, $NaHCO_3$ (iv) 1,4-dioxane 120-130 ^{o}C, 8-9h, K_2CO_3

Figure 1. SAR of hybrid 4-aminoquinoline 1,3,5-triazine derivatives

Figure 2.Docking pose of hybrid compound **8g** in the active site of wild *pf*-DHFR-TS. (Ligand stick colored by atom type, where, Grey: C, Green: Cl, Blue: N, White: H, while the vital neighboring amino acid residues were colored according to interpolated charges.)

Figure 3.Docking pose of compound **8g** in the active site of qradruple mutant type *pf*-DHFR-TS. (Ligand stick colored by atom type, where, Grey: C, Green: Cl, Blue: N, White: H, while the vital neighboring amino acid residues were colored according to interpolated charges.)

Table 1: *In vitro* antimalarial activity of Hybrid derivatives **8(a-i)**.

Compounds	*% dead asexual parasites*			
	3D-7[*]		*RKL-2*[#]	
	[$]5µg/ml	[$]50µg/ml	[$]5µg/ml	[$]50µg/ml
8a	5.0	25.5	20.3	38.3
8b	20.5	16.0	19.6	36.6
8c	6.5	8.0	20.6	34.6
8d	6.5	13.5	16.0	36.6
8e	11.5	29.0	15.0	28.0
8f	5.0	18.0	18.3	40.6
8g	22.0	36.0	22.3	45.3
8h	19.5	26.5	9.6	22.0
8i	9.5	14.0	14.3	34.6
Chloroquine (0.7µg/ml)	49.5		-	
Chloroquine (1.2µg/ml)	-		45.0	
Proguanil (>200µg/ml)	50		50	

**- Wild P. falciparum parasite (Chloroquine sensitive)*

#- Mutant P. falciparum parasite (Chloroquine resistant)

$- Test dosage

Table 2: *In vivo* antimalarial evaluation of representative lead compound against *P. berghei* in swiss albino mice model

Treatment	Mean % parasitaemia inhibition	Mean survival time (MST) of treated mice (days ± SD)
Chloroquine	90.35	22.09 ± 9.22
8g	64.50	21.3 ± 4.04

Dosage 5mg/kb/day

Table 3: Docking interaction of hybrid derivatives **8(a-i)** in wild and qradruple mutant *pf*-DHFR-TS

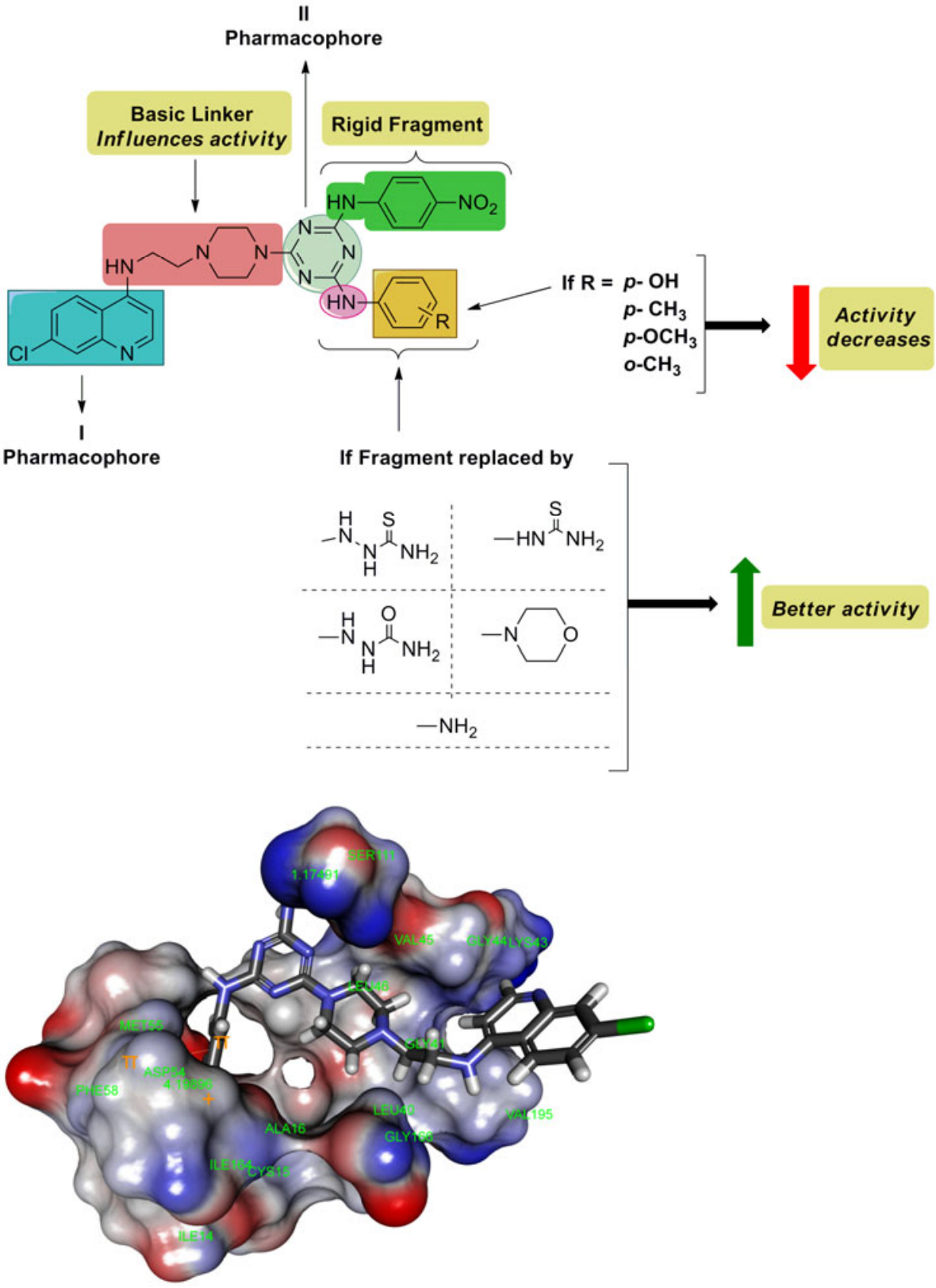
Compound	Wild type *pf*-DHFR-TS			Qradruple mutant *pf*-DHFR-TS		
	Donor/acceptor Hydrogen bond	*Non-bonded forces*	*Binding energy(Kcal/mol)*	*Donor/acceptor hydrogen bond*	*Non-bonded forces*	*Binding energy (Kcal/mol)*
8a	Quinoline-NH….. SER167 Quinoline ring-N….. SER108	π-σ Quinoline —THR107 π-+ Phenyl NO— PHE58	-72.45	Quinoline-NH….. SER167	-	-35.54
8b	1,3,5-triazine-phenyl-NH……SER 111	π-π Phenyl— PHE58	11.27	1,3,5-triazine-phenyl-NO…….ALA16 Quinoline-NH….. LYS49	π-π Phenyl— PHE116 π-+ Phenyl NO-----PHE58	-85.11
8c	1,3,5-triazine-phenyl- NH….ILE164 1,3,5-triazine	π-π Phenyl— PHE58	-44.44	1,3,5-triazine-Phenyl NO…… SER120, ARG159,	π-π Phenyl— PHE116 π-σ	-81.41

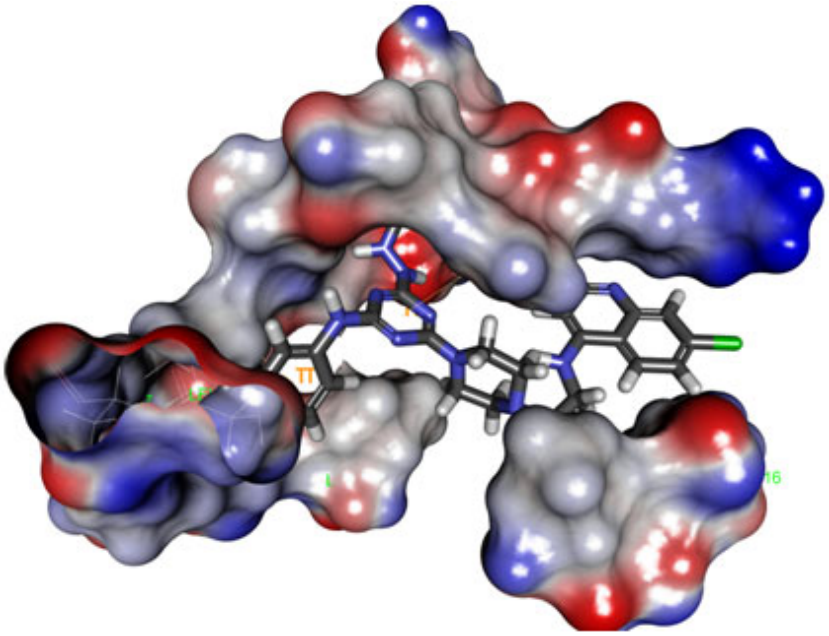
	Phenyl OH….ASP54			ARG122 1,3,5-triazine Phenyl OH….LYS49	Quinoline Piperazine-PHE58	
8d	1,3,5-triazine-phenyl NH….SER111	π-π Phenyl—PHE58 π-+ Phenyl NO—PHE58	-47.28	1,3,5-triazine-Phenyl NO…….. ARG59	π-+ Phenyl—ARG122	-110.55
8e	Quinoline-NH….. SER167 1,3,5-triazine – phenyl-NH…..ILE164 1,3,5-triazine-Thiosemicarbazie-NH2…..ILE164, TYR170	-	-39.77	1,3,5-triazine-NH…TYR170 1,3,5-triazine-NH…ASP54 1,3,5-triazine thiosemicarbazide-NH2…..THR185	π-π 1,3,5-triazine—PHE58 π-σ Quinoline –ARG59	2.00
8f	1,3,5-triazine-thiourea NH2……TYR170	π-σ Triazine—LEU46	-39.65	1,3,5-triazine-Phenyl NH……SER111 1,3,5-triazine-thiourea NH……SER111	π-+ Quinoline---ARG59 π-+ phenyl-NO---PHE58	-21.77
8g	1,3,5-triazine-phenyl NH……SER111	π-π Phenyl—PHE58 π-+ Phenyl NO---PHE58	4.74	1,3,5-triazine-phenyl NO……SER167 1,3,5-triazine-phenyl NH…TYR170 1,3,5-triazine semicarbazide-NH…..ASP54 1,3,5-triazine-semicarbazide-NH2….CYS15	π-π 1,3,5-triazine—PHE58 π-+ Phenyl-NO---LEU40	-9.66
8h	1,3,5-triazine-NH ……SER167	π-π Phenyl—	72.74	1,3,5-triazine-phenyl	π-+ Quinoline--	-84.21

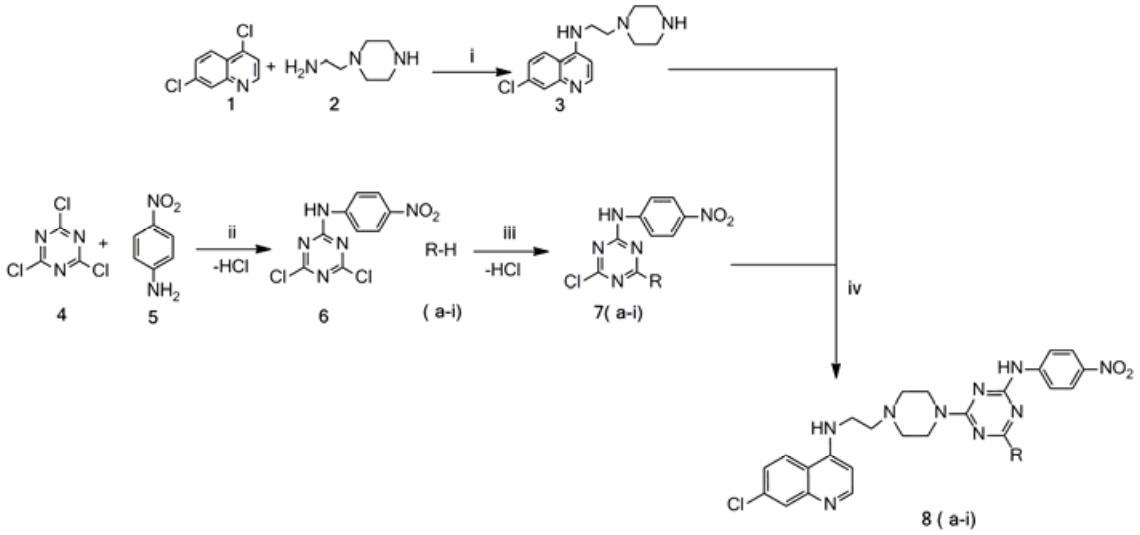
	1,3,5-triazine NH…..SER111	PHE58 π-σ Phenyl NO— PHE58		NH…SER111	-ARG59		
8i	-	π-π Phenyl— PHE58 π-+ Phenyl NO---PHE58	18.27	Quinoline ring N…..SER120 1,3,5-triazine-phenyl NH…SER111	π-+ Quinoline---ARG59, ARG122	-35.43	

Table 4: Docking scores of compound **8g** and **8c**

	Wild type *pf*-DHFR-TS				**Quadruple mutant *pf*-DHFR-TS**			
Scoring parameter	Dock Score	-PMF	-PLP1	-PLP2	Dock Score	-PMF	-PLP1	-PLP2
8g	70.25	65.78	111.99	94.73	141.90	144.52	150.9	140.42
8c	68.92	57.68	103.84	92.72	76.68	121.82	88.32	97.82







Where R=