Pharmacological Reports 71 (2019) 762–767

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original article

In silico ADMET study, docking, synthesis and antimalarial evaluation of thiazole-1,3,5-triazine derivatives as *Pf*-DHFR inhibitor

Supriya Sahu[a], Surajit Kumar Ghosh[a], Prashant Gahtori[b], Udaya Pratap Singh[c], Dibya Ranjan Bhattacharyya[d], Hans Raj Bhat[a,*]

[a] *Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India*
[b] *School of Pharmacy, Graphic Era Hill University Dehradun, Uttarakhand, India*
[c] *Drug Design and Discovery Laboratory, Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture Technology and Sciences, Allahabad, India*
[d] *Regional Medical Research Centre, ICMR, Dibrugarh, Assam, India*

ARTICLE INFO

Article history:
Received 20 September 2018
Received in revised form 12 March 2019
Accepted 8 April 2019
Available online 19 April 2019

Keywords:
Thiazole
1,3,5-Triazine
Antimalarial
ADMET
Docking
Synthesis

ABSTRACT

Background: Plasmodium falciparum dihydrofolate reductase (*Pf*-DHFR) is an essential enzyme in the folate pathway and is an important target for antimalarial drug discovery. In this study a modern approach has been undertaken to identify new hits of thiazole-1,3,5-triazine derivatives as antimalarials targeting *Pf*-DHFR.

Methods: The library of 378 thiazole-1,3,5-triazines were designed and subjected to ADME analysis. The compounds having optimal ADME score, was then evaluated by docking against wild and mutant *Pf*-DHFR complex. The resultant compound after screening from above these two methods were synthesized, and assayed for *in vitro* antimalarial against chloroquine-sensitive (3D-7) and chloroquine resistant (Dd-2) strains of *P. falciparum*.

Results: Twenty compounds were identified from the dataset based on considerable AlogP98 *vs.* PSA_2D confidence ellipse, ADME filter and TOPKAT toxicity analysis. Majority of compounds showed interaction with Asp54, Arg59, Arg122 and Ile 164 in docking analysis. Entire set of tested derivatives exhibited considerable activity at the tested dose against sensitive strain with IC_{50} values varying from 10.03 to 54.58 μg/ml. Furthermore, against chloroquine resistant strain, eight compounds showed IC_{50} from 11.29 to 40.92 μg/ml. Compound **A5** and **H16** were found to be the most potent against both the strains of *P. Falciparum*.

Conclusion: Results of the study suggested the possible utility of thiazole-1,3,5-triazines as new lead for identifying new class of *Pf*-DHFR inhibitor.

Introduction

Despite the enhanced research funding over the years, malaria is responsible for 0.435 million deaths worldwide in 2017. Five less developed-regions Nigeria, Democratic Republic of the Congo, Mozambique, India and Uganda accounted for nearly half of all malaria cases reported worldwide [1,2]. According to an estimate, malaria currently accounts for one of the largest drug markets in the world [3,4]. The malaria parasites have acquired resistance against nearly all antimalarial drugs, and posing greatest threat to the malaria control [5,6]. The artemisinin discovery was sought as a very powerful and efficacious intervention; however, the emergence of resistance against artemisinin has seriously compromised its clinical utility [7,8]. Therefore, the research and development of new antimalarial agents are vital for malaria control [9]. The structure-based drug discovery efforts provide better access to innovation and also reduce manpower to identify cost-effective therapeutic agents [10]. *Plasmodium falciparum* DHFR crystal structure is a major drug target and several computational techniques have been applied successfully to identify new and effective DHFR inhibitors [11,12].

In continuation of our interest in hybrid thiazole-1,3,5-triazine derivatives [13–15], in-house library consisting of 378 conjugates from thiazoles and 1,3,5-triazine class was designed. The compounds were filtered by ADMET profiling and ranked-order through the structure-based model. Finally, twenty top rank-ordered compounds were selected for synthesis and biological screening using 3D-7 (chloroquine sensitive) and Dd2 (chloroquine resistant) strains of

* Corresponding author.
E-mail address: pharmahans@gmail.com (H.R. Bhat).

http://dx.doi.org/10.1016/j.pharep.2019.04.006
1734-1140/

P. falciparum. Additionally, docking was applied to expose necessary key structural features of the potential DHFR inhibitors.

Material and methods

ADMET study

The library of 378 compounds and two reference drugs WR99210/ Pyrimethamine was selected for calculating the ADMET descriptors (absorption, distribution, metabolism, excretion and toxicity) using Discovery studio 3.1 (Accelrys, San Diego, CA, USA). ADMET descriptors protocol was utilised to predict descriptors Absorption, Solubility, Hepatotoxicity, Plasma protein binding (PPB), AlogP98 and PSA_2D values (Supplementary file). The model for plotting the confidence ellipse was developed by Egan and Lauri [18] with descriptors that include AlogP98 and 2D polar surface area (PSA_2D). Aqueous solubility was predicted by using a model developed by Cheng and Merz [19] with $R^2 = 0.84$. All the models used for predicting the ADME and toxicity have high R^2 values (Supplementary file) and for plotting confidence ellipse (Fig. 2). Compounds which were found outside the 95% and 99% ellipse region were poorly absorbed compounds (< 30% absorbed). The upper limit of PSA_2D value and AlogP98 for the 99% confidence ellipsoid was 148.12 and 6.5 respectively. Compounds with higher values than 148.12 of PSA_2D and more than 6.5 for AlogP98 were eliminated from the study. Thus the library had shrunk the compounds from 378 to 251. Moreover, compounds with low (2) and very low (3) human intestinal absorption, extremely low (0) or no drug likeness (5), hepatotoxicity (1) and < 90% plasma protein binding (0) were eliminated. Among the reference compounds WR99210 and Pyrimethamine, WR99210 showed hepatotoxicity whereas Pyrimethamine successfully crossed all the parameters. WR99210 was well known for its withdrawal from clinical trials because of toxicity problems. So, it can be said that the protocol of ADMET filter used in this study was a validated one. The designed library had again shrunk from 251 to 51 compounds which were taken for prediction of carcinogenicity again. From the results of TOPKAT toxicity prediction it has been seen that out of the 51 compounds, 15 showed either carcinogenicity in any one model or Ames mutagenicity and 16 compounds had very low (<100 mg/kg) rat oral LD50 values (Supplementary file). WR99210 had shown carcinogenicity in both male and female mouse model which further validates the protocol. Thus the library of 51 compounds had ultimately shrunken to 20 compounds.

Docking

Docking was performed on the crystal structure of wild and mutant *Pf*-DHFR complex obtained from Protein Data Bank (1j3i and 1j3k). In the protein workspace of Accelrys's DS v3.1, water molecules, co-crystallized ligand WR99210 were removed and cofactors NADPH, dUMP were retained. *Pf*-DHFR consist of four chains A, chain B, chain C and chain D, where chain C and chain D are of TS domain and chain A and chain B corresponds to DHFR domain. Since the prototype WR99210 was bound to chain A, thus only chain A of the protein was used in the present work. This refined protein was simulated in the workspace by applying CHARMm forcefield and finally binding site was defined as sphere (28.00, 5.89121, 59.83, 16.10) around the active site of chain A. Docking was done using CDOCKER of Accelrys's DS v3.1. The centre of co-crystallized ligand WR99210 was selected as the binding site for all calculations. The protocol was validated by calculating RMSD between five docked poses of WR99210 and ligand's X-ray docking pose. The orientation of WR99210 was taken as reference.

Chemistry

All the chemicals and solvents used for synthesis, recrystallization and analysis were of AR grade and used without further purification. Melting point of the synthesized compounds was determined by Melting Point apparatus (BUCHI Melting Point M560) at 10 °C/min temperature gradient. The UV-Spectra (λ_{max}) of the synthesized compounds were recorded on Shimadzu, UV-1800, UV-VIS spectrophotometer instrument. The FT-IR spectra of the synthesized compounds were recorded on Bruker ALPHA FTIR spectrometer. The ^{1}H-NMR spectra of the synthesized compounds were recorded in DMSO at 300 MHz by Bruker Avance DPX 300 NMR spectrometer and ^{13}C-NMR was also recorded in DMSO at 100 MHz by Bruker Avance DPX 100 NMR spectrometer. The mass spectra of the synthesized compounds were recorded on ZQ-4000 equipped with an Electrospray Ionizer as an ionization method.

Synthesis of the intermediates (**3**) and (**5**) were achieved by earlier reported procedures shown in Scheme 1 [13,16].

General procedure for the synthesis of title compounds

Different aliphatic or aromatic amine (0.02 mol) was added to product (**5**, 0.01 mol) obtained in second step. Reaction was carried out in microwave at temperature 120 °C, pressure:10 bar, power: 100 W, time: 9 min. After the reaction, the resulting solid was filtered, washed with water and dried to afford final derivatives. The title compounds were characteriazed by FT-IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectroscopic analysis (Supplementary file).

In vitro *antimalarial activity screening*

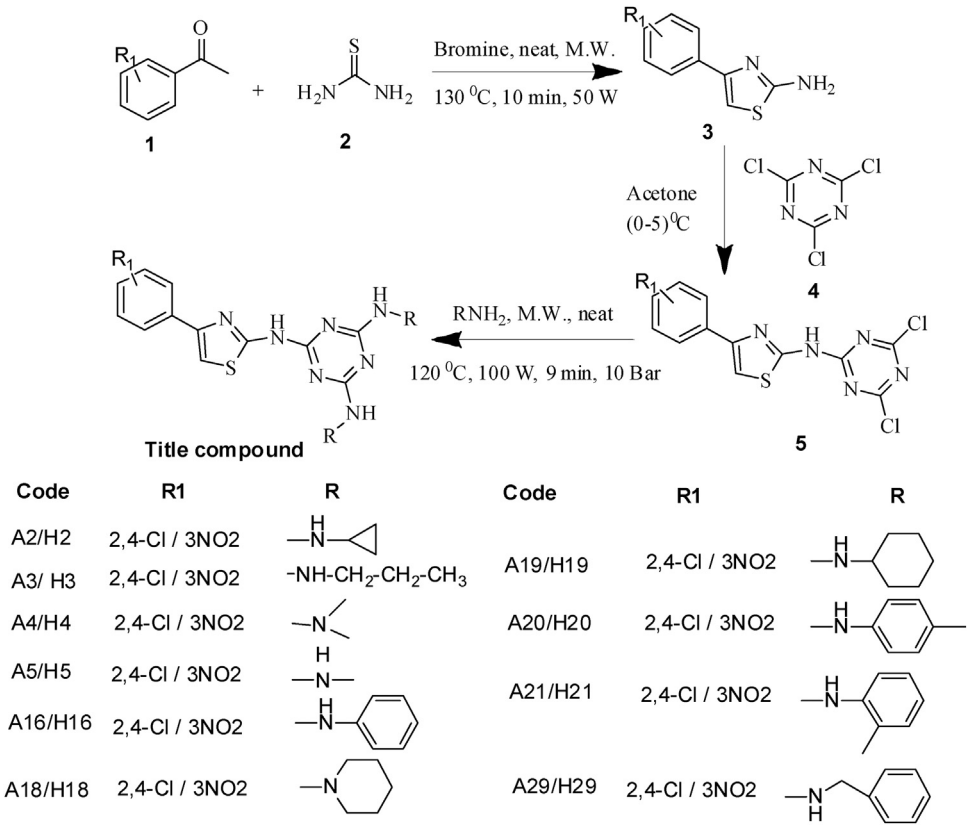
Chloroquine sensitive and resistant strains of *P. falciparum* namely 3D7 and Dd2 were maintained routinely in stock cultures in medium RPMI-1640 supplemented with 25 mmol 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 1% D-glucose, 0.23% sodium bicarbonate, and 10% heat inactivated human serum. The asynchronous parasites of *P. falciparum* 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 0.8–1.5% at 3% hematocrit in a total volume of 200 μl of medium RPMI-1640 was uniformly maintained.

The *in vitro* antimalarial assay was carried out according to microassay of Rieckmann et al. in 96 well microtitre plates, with minor modifications [17]. A stock solution of 5 mg/ml of each of the test samples was prepared in dimethyl sulfoxide (DMSO) and subsequently diluted with the culture medium. The test compounds in 20 μl volume concentration at 5 μg/ml and 50 μg/ml in a duplicate well were incubated with parasitized cell preparation at 37 °C in a candle jar. After 36–40 h of incubation, the blood smears were prepared from each well and stained with Giemsa stain. The level of parasitemia in terms of % dead rings along with trophozoites and schizonts was determined by counting a total of 100 asexual parasites (both live and alive) microscopically using chloroquine as the reference drug.

Results and discussion

ADMET study

A library of 378 substituted phenylthiazole 1,3,5-triazine derivatives were designed based on the structural requirement of existing antimalarial-antifolates and subjected to ADMET study. A total 49 compounds have shown good human intestinal



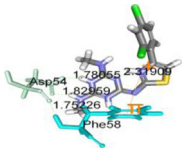
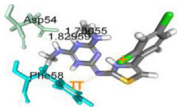
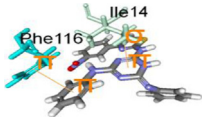
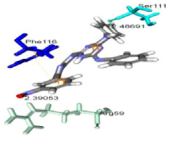
Scheme 1. Synthetic protocol of the final compounds.

absorption (absorption level 0) with two reference drugs WR99210/ Pyrimethamine. 81 compounds have shown moderate absorption, 233 have shown low absorption and remaining compounds showed very low absorption. On investigating aqueous solubility, it was found that 111 compounds showed extremely low aqueous solubility, 172 with very low and 98 showed considerable aqueous solubility. 241 compounds showed hepatotoxicity scores to 1 and eliminated due to probable toxic effect. 139 compounds found AlogP98 value less than five to fall under good absorption. Moreover, 67 compound had shown plasma protein binding <90%, 61 with binding ≥90% and 250 compounds have plasma protein binding ≥95% indicating that majority of compounds have high probability to reach the desired targets. Twenty compounds were selected on basis of AlogP98 *vs.* PSA_2D confidence ellipse, ADME filter and TOPKAT toxicity prediction.

Docking

The docking studies of selected 20 compounds were performed in the binding pocket of both the wild type (1j3i.pdb) and quadruple mutant (1j3k.pdb) *pf*-DHFR (Table 1). Docking study showed compound **A5** with lowest binding energy and strong H-bonded interactions with Asp54 with one weak pi-cationic interaction with Phe58 in both wild and quadruple mutant *pf*-DHFR. Compound **H16** had shown pi-pi interactions with Phe116 and pi-sigma interaction with Ile14 of 1j3i, whereas with 1j3k, it interacted with Phe116, Ser111 and Arg59 through pi-pi and H-bond interaction. Against both the protein, it showed almost same amount of binding energy which was found second lowest out of the twenty tested compounds. Compound **A3** showed pi-cationic interaction with Phe58 and H-bonding with Ser 111 in both the wild and mutant forms of *Pf*-DHFR with equal amount of binding energy. Compounds **A5**, **H16** and **A3** were scored as the first, second and third highest by LigScore1_dreiding in both the wild and mutant forms of *Pf*-DHFR. Compound **A2** had not shown any interaction in both the targets, but still had a good score in LigScore1_dreiding. The stability of the conformer of **A2** inside the binding pocket of the protein might be the reason for its seventh highest score among the twenty compounds. From the docked poses and binding energies of compounds **A2**, **A3** and **A5,** it was evident that compounds with same docked pose had similar binding energies in both wild (1j3i) as well as in mutant (1j3k) variety of *Pf*-DHFR. The fourth, fifth, sixth and eighth position in the scoring of LigScore1_dreiding for 1j3i were occupied by compounds **H2**, **A29**, **H18** and **A21,** whereas for 1j3k the order is simply reversed. The presence of Arg122 in the binding pocket of compound **A21**, Arg59 in the binding pocket of compound **H18**, both Arg122 and Arg59 in the binding pocket of compound **H2** and

Table 1
Docking poses in the active site of 1j3i and 1j3k.

Compound	1j3i	1j3k
A5		
H16		

Leu46 in the binding pocket of compound **A29** which were the key amino acid of mutation might be the reason of this reversal. Compounds **H4** and **H19** had not shown any interactions with 1j3i like compound **A2** but had a good score.

The involvement of the key amino acids in the binding pocket of top two compounds **A5** and **H16** was validated by a comparison of their docking poses with antimalarial antifolates like WR99210, compounds **12b**, **29d**, **34d** and **21c** of same hybrid [20]. The interaction with the same amino acids, for instance, Asp54, Arg59, Arg122, Ile 164 validates the results of the present study. Some hits like **A5** and **H16** that showed interaction with some amino acids which were the key points of mutation. This was well described by their comparable activity against the chloroquine resistant strain of *P. falciparum* Dd2.

Compounds **A4**, **A16**, **A18**, **A19**, **A20**, **H3**, **H4**, **H5**, **H19**, **H20**, **H21** and **H29** were not been able to fit into the binding pocket of 1j3k, so no binding energy was generated followed by no interaction and no dock score (Supplementary file).

Chemistry

The synthesis of 20 compounds were accomplished by the nucleophilic substitution reaction of the chlorine atoms of 1,3,5-triazine by 2-amino-4-(substituted phenyl)thiazole with different aliphatic, aromatic amines. The first chlorine of 1,3,5-triazine was substituted with previously 2-amino-4-(substituted phenyl)thiazole in acetone at 0–5 °C [13]. Second and third chlorine were replaced with different amines at 120 °C under microwave irradiation. The completion of the reaction was ascertained on the basis of TLC (Scheme 1).

UV wavelength of maximum absorbance of this series of compounds was found to be at about 250 nm due to n- π^* transition. Characteristic FT-IR peaks were observed in the region 1500–1230 cm^{-1} due to —C=C—, —C=N— stretching. ^{1}H-NMR of the compounds showed a singlet in between at δ 4.4–4.6 due to —NH group of the hybrids. Peak observed in between 4.2-3.3 was due to aromatic C—NH. Disappearance of the peaks corresponding to primary amine associated with 2-amino-4-(substituted phenyl) thiazole further confirmed the successful synthesis of this class of compounds. ^{13}C-NMR showed the appearance of carbon signal at about 160–180 ppm. Isolation of pure products was facilitated by simply washing with water. This was due to low solubility of the synthesized organic compounds in water.

In vitro *antimalarial activity screening*

The antimalarial activities of the synthesized compounds were determined first in terms of % dead rings, trophozoites and schizonts followed by IC_{50} determination (Table 2). The *in vitro* antimalarial activity of the final twenty compounds of this study was used to revalidate the scoring function LigScore1_dreiding. This was done by plotting a graph and calculating the R^2 value between the IC_{50} data and the scoring function LigScore1_dreiding (Fig. 1). The sufficiently high R^2 value of 0.94 and 0.92 in choroquine sensitive 3D7 and chloroquine resistant Dd2 strain respectively revalidated the scoring function LigScore1-drieding used in the present study. Compounds **A5**, **H16** and **A3** which were scored as the first, second and third highest by LigScore1_dreiding had also the similar pattern of lowest IC_{50} values in both the wild and mutant forms of *Pf*-DHFR.

The results suggested that the presence of Arg122 in the docked pose of compound **A21** with mutant (1j3k) ensured its good activity against chloroquine resistant Dd2 strain. On the other hand, the presence of Leu46 in the binding pocket of compound **A29** with mutant (1j3k) might be the reason of its almost doubled binding energy. The presence of Arg59 in all the three compounds **H2**, **H16** and **H18** in the binding pocket of mutant (1j3k), might be the reason of their higher activity. Moreover, the presence of Arg122 in the binding pocket of compound **H2** in mutant (1j3k) ensures its better activity.

Compounds having secondary amino substitutions like in **A3**, **A5** and **H16** had shown highest percentage of dead rings, schizonts and trophozoites. After that compounds with substitution of cyclopropylamine (**A2**, **H2**), piperidine (**H18**) and benzylamine (**A29**) had second highest *in vitro* activity. Compounds with aromatic secondary amines as in **A20** and **A21** had the next highest percentage of deaths. Whereas compounds having tertiary amine like **A4** and **A18** were found to be less active. Whereas, the cyclopropylamine substitution as in the case of **H2** showed highest percent dead rings, schizonts and trophozoites at lower 5 μg/ml dose level. Therefore, the importance of secondary amines cannot be overlooked. It was also observed that compounds with more

Table 2
In vitro antimalarial activity of the compounds against 3D7 and Dd2 strain.

Compd Code	Percent dead rings, trophozoites and schizonts against 3D7			Percent dead rings, trophozoites and schizonts against Dd2		
	5 μg/ml	50 μg/ml	IC_{50}	5 μg/ml	50 μg/ml	IC_{50}
A2	24	80.5	23.33	5	22	31.8
A3	55.5	90	12.48	22.5	57	15.73
A4	23.5	35	35.74	–	–	–
A5	20	100	10.03	22	67	11.29
A16	23	28	38.29	–	–	–
A18	0	38	38.48	–	–	–
A19	21	45	30.95	–	–	–
A20	14	71	28.93	–	–	–
A21	26	77.5	25.93	13	52	18.99
A29	22	84	18.37	16	27	25.94
H2	69	85	15.2	0	21	40.92
H3	0	15.5	45.76	–	–	–
H4	13	26	41.44	–	–	–
H5	16.5	40.5	32.56	–	–	–
H16	26.5	97.5	11.34	23	59	12.55
H18	16	82.5	20.38	18	48	17.74
H19	12.5	40.5	33.07	–	–	–
H20	30	43	32.91	–	–	–
H21	0	8	54.58	–	–	–
H29	6	45.5	33.75	–	–	–
Chloroquine (standard)			0.7			1.2

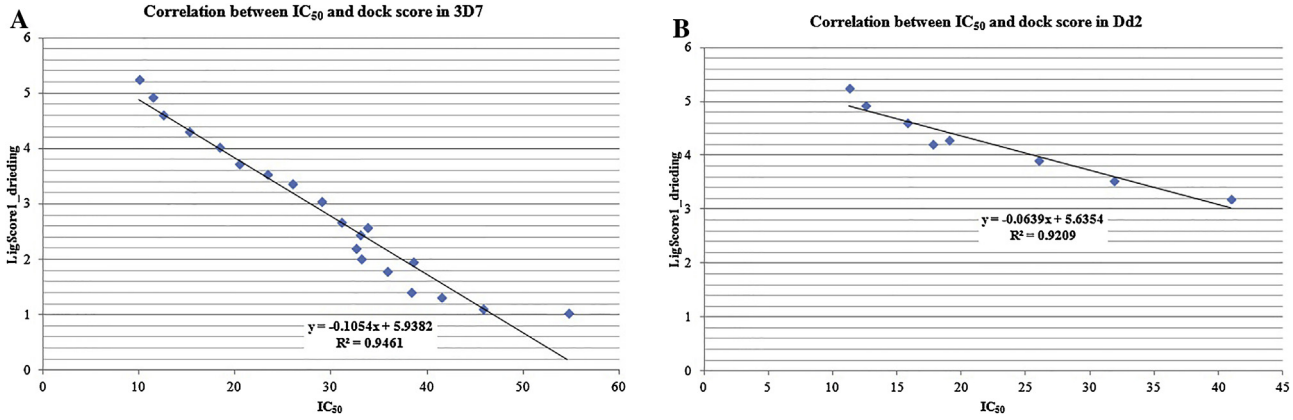


Fig. 1. Relationship between IC_{50} and LigScore1_drieding in (a) 3D7 and (b) Dd2.

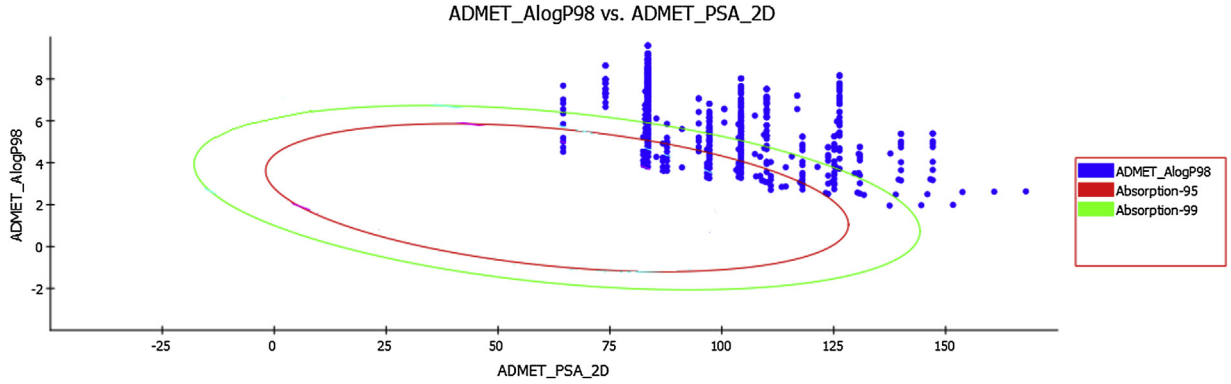


Fig. 2. 95% and 99% confidence ellipses of human intestinal absorption.

number of secondary nitrogen exhibiting + I-effect were the most active ones.

Conclusion

In conclusion, the present study shown the synthesis of hybrid thiazole-1,3,5-triazines and their antimalarial evaluation against choroquine sensitive 3D7 and chloroquine resistant Dd2 strain of *P. falciparum*. The result suggested, that compounds **A5** and **H16** as most potent derivatives among the tested compounds with significant *in vitro* antimalarial activity and may serve as lead for identifying new class of *Pf*-DHFR inhibitor.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

The financial support from the Department of Biotechnology (DBT), New Delhi, India is gratefully acknowledged [grant no. BCIL/NER-BPMC/2013-541 (BT/330/NE/TBP/2012 dated 29 April 2013)] and the authors also are thankful to S.A.I.F., Punjab University, Chandigarh, India for providing spectroscopic data.

References

[1] WHO fact-sheets on malaria. 2018. (Assessed 22 December 2018) https://www.who.int/news-room/fact-sheets/detail/malaria.
[2] Lichtenberg FR. Pharmaceutical innovation and the burden of disease in developing and developed countries. New York: Columbia University and the National Bureau of Economic Research; 2005.
[3] Craft JC. Challenges facing drug development for malaria. Curr Opin Microbiol 2008;11(5):428–33.
[4] White MT, Conteh L, Cibulskis R, Ghani AC. Costs and cost-effectiveness of malaria control interventions—a systematic review. Malar J 2011;10:337.
[5] Price RN, von Seidlein L, Valecha N, Nosten F, Baird JK, White NJ. Global extent of chloroquine-resistant Plasmodium vivax: a systematic review and meta-analysis. Lancet Infect Dis 2014;14:982–91.
[6] Hanboonkunupakarn B, White NJ. The threat of antimalarial drug resistance. Trop Dis Travel Med Vaccines 2016;2:10.
[7] Anvikar AR, Sharma B, Sharma SK, Ghosh SK, Bhatt RM, Kumar A, et al. *In vitro* assessment of drug resistance in Plasmodium falciparum in five States of India. Indian J Med Res 2012;135:494–9.
[8] Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, et al. Artemisinin resistance in Plasmodium falciparum malaria. N Engl J Med 2009;361:455–67.
[9] Ashley EA, Phyo AP. Drugs in development for malaria. Drugs 2018;78(9):861–79.
[10] Schuhmacher A, Gassmann O, Hinder M. Changing R&D models in research-based pharmaceutical companies. J Transl Med 2016;14:105.
[11] Nzila A. Inhibitors of de novo folate enzymes in Plasmodium falciparum. Drug Discov Today 2006;11:939–44.
[12] Toyoda T, Brobey RK, Sano G, Horii T, Tomioka N, Itai A. Lead discovery of inhibitors of the dihydrofolate reductase domain of Plasmodium falciparum dihydrofolate reductasethymidylate synthase. Biochem Biophys Res Commun 1997;235:515–9.
[13] Sahu S, Ghosh SK, Ghoshal A, Kalita J, Gahtori P, Bhattacharyya DR. Microwave assisted synthesis, antimalarial screening and structure–activity-relationship exploration of some phenylthiazolyl-triazine derivatives against dihydrofolate reductase. Med Chem Res 2016;25:2916–23.
[14] Sahu S, Ghosh SK, Kalita JM, Dutta M, Bhat HR. Design, synthesis and antimalarial screening of some hybrid 4-aminoquinoline-triazine derivatives against pf-DHFR-TS. Exp Parasitol 2016;163:45–9.
[15] Gahtori P, Pandey R, Kumar V, Ghosh SK, Das A, Kalita JM, et al. Toward resistance-compromised DHFR inhibitors part 1: combined structure/ligand-based virtual screenings and ADME-Tox profiling. J Chemom 2016;30:462–81.

[16] Reaume AG. Drug repurposing through nonhypothesis driven phenotypic screening. Drug Discov Today Ther Strateg 2012;8:85–8, doi:http://dx.doi.org/10.1016/j.ddstr.2011.09.007.
[17] Rieckmann KH, Sax LJ, Campbell GH, Mrema JE. Drug sensitivity of P. falciparum: an *in vitro* microtechnique. Lancet 1978;311:22.
[18] Egan WJ, Lauri G. Prediction of intestinal permeability. Adv Drug Del Rev 2002;54:273–89.
[19] Cheng A, Merz K. Prediction of aqueous solubility of a diverse set of compounds using quantitative structure-property relationships. J Med Chem 2003;46:3572–80.
[20] Gahtori P, Ghosh SK, Parida P, Prakash A, Gogoi K, Bhat HR, et al. Antimalarial evaluation and docking studies of hybrid phenylthiazolyl-1,3,5-triazine derivatives: A novel and potential antifolate lead for Pf-DHFR-TS inhibition. Exp Parasitol 2012;130:292–9.