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ARTICLE

Microwave assisted synthesis, docking and antimalarial evaluation of hybrid PABA-substituted 1,3,5-triazine derivatives

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Abstract

A series of novel PABA-substituted 1,3,5-triazine derivatives were developed via microwave assisted synthesis and subsequently tested for antimalarial activity against chloroquine sensitive 3D7 strain of *Plasmodium falciparum* using chloroquine as standard. Antimalarial screening result showed that synthesized compounds exhibited IC_{50} in the range of 4.46 to 79.72 μg mL^{-1}. Among the tested compounds, **4c** and **4f** showed significant antimalarial activity with low binding energies (BE) -172.32 and 160.41 kcal mol^{-1} via interacting with Arg122 through the involvement of COOH of the phenyl linked to 1,3,5-triazine. In conclusion, these core scaffolds can be used for future antimalarial drug development.

1 | INTRODUCTION

Malaria is a growing infectious disease and becoming a serious health concern around the developing world. The current data on the incidence and mortality of malaria shows that 90 countries especially from Africa, South-East Asia and Eastern Mediterranean region are majorly affected by it.[1] The disease affects around 219 million people globally and accounted for almost 435 000 deaths in 2017 alone.[2] The mutant form of *Plasmodium falciparum* currently poses significant risk to currently available antimalarial agents.[3–6] Therefore, it is worthwhile to discover newer agents which can be able to arrest the growth of parasites.

Modified 2,4,6-trisubstituted 1,3,5-triazine derivatives offer access to a diverse array of useful molecules[7] which demonstrated wide range of therapeutic activities, such as, antifungal,[8] antibacterial[9,10] and anti-HIV,[11] anti-diabetic, anti-inflammatory, antitumor and against cystic-fibrosis.[12,13] Moreover, substituted 1,3,5-triazine derrivatives remain the attractive scaffolds for the generation of antimalarial drugs, such as, cycloguanil, chlorcycloguanil, clociguanil and WR99210 (diaminotriazine).[14–16]

1,3,5-Triazines have been identified as non-classical antifolates and resulted in many potent DHFR inhibitors. In crystallographic studies of WR99210 with *M tuberculosis* DHFR or human DHFR with their cofactor NADPH suggested that the triazine core oriented as a pseudo chair confirmation with C2 moving out of the plane of the ring. The two amino groups form four hydrogen

bonds with protein residues Ile5, Asp27, Ile94 and Tyr100, which is similar to the binding between triazines with chicken liver DHFR.[17] This leads to the discovery of various 1,3,5-triazine derivatives as potent inhibitors of DHFR against malaria as well as cancer. On the other hand, numerous sulphonamides and sulphones were identified as potent antifolate antimalarial drugs belonging to the category of *p*-amino benzoic acid (PABA), an essential precursor for the de novo synthesis of folic acid.[18] However, the clinical utility of above moieties against malaria parasite is jeopardized due to drug resistance, poor pharmacokinetics and toxic side-effect. Concerning this and previous studies which suggests hybridization of two distinct moieties improve pharmacokinetics as well as pharmacological activity via dual action,[19] we intended to develop novel series of PABA-substituted 1,3,5-triazine derivatives was developed via microwave assisted synthesis and subsequently tested for antimalarial activity against 3D7strain of *P falciparum* (*Pf*). The molecules were also docked in the active site of *P falciparum* dihydrofolate reductase thymidylate synthetase (*Pf*-DHFR-TS) to explain the interaction vital for bioactivity generation.

2 | RESULTS AND DISCUSSION

2.1 | In-silico screening and docking

The compounds **4**(**a-j**) were analyzed through preliminary screening filters and the results were presented in Tables 1 and 2. All the title compounds followed Lipinski's rule of five reflecting good oral bioactivity and indicated no risk in case of carcinogenicity, Ames mutagenicity and skin irritancy. The ADMET screening results showed all the test compounds have good human intestinal absorption.

Entire compounds showed binding energies within the range of −153.10 to −224.14 (kcal mol^{-1}) which found greater than the binding energy of WR99210, the originally bound ligand with 1J3K (−144.15 kcal mol^{-1}) as shown in Table 3. Docking results showed that synthesized compounds have considerable and diverse binding affinities against mutant type (1J3K pdb) *Pf*-DHFR-TS via formation of H bonds, pi-pi and pi-sigma interactions as shown in Figure 1. Compounds **4c** and **4f** showed higher antimalarial activity with low binding energies −172.32 and −160.41 (kcal mol^{-1}) respectively due to the formation of H bond with Arg122 on interacting with COOH group of phenyl ring linked to 1,3,5-triazine. Along with compound **4h** showed sigma-cation interaction with COOH group at phenyl linkage with 1,3,5–triazine ring. Compounds **4d**, **4e**, **4i** and **4j** showed pi-pi interaction with Arg122 through the involvement of COOH group attached on phenyl linkage with 1,3,5-triazine. Furthermore, compounds **4a** and **4d** showed sigma-pi interaction with the involvement of 1,3,5-triazine ring. Compounds **4d**, **4e** and **4i** showed mild to moderate activity by forming H bond with Phe58 through COOH group of phenyl linkage with 1,3,5-triazine ring. All the synthesized compounds showed strong H-bond interaction with Arg122, which was suggested to be responsible for the generation mutation in *Pf*-DHFR-TS.[20]

2.2 | Chemistry

The synthesis of the title compounds was achieved as shown in Scheme 1. Initially, the synthesis was started with the development of 4,6-dichloro-1,3,5-triazine-2-amine (**2**)

TABLE 1 Predicted molecular properties of the compounds using "Molinspiration cheminformatics" software

Compound code	mi LogP	TPSA	No. of atoms	Molecular weight	No. of H-bond acceptor	No. of H-bond donor	No. of violation	No. of rotatable bonds	Volume
4a	1.93	126.05	19	260.26	8	5	0	4	222.64
4b	2.81	126.05	21	288.31	8	5	0	6	256.24
4c	3.37	126.05	22	302.34	8	5	0	7	273.04
4d	3.76	126.05	25	340.32	8	5	0	5	282.42
4e	3.81	126.05	25	340.32	8	5	0	5	282.42
4f	4.45	126.05	25	401.22	8	5	0	5	297.37
4g	4.07	126.05	25	336.36	8	5	0	5	294.05
4h	4.09	126.05	25	336.36	8	5	0	5	294.05
4i	4.04	126.05	25	336.36	8	5	0	5	294.05
4j	4.30	126.05	25	356.77	8	5	0	5	291.02

TABLE 2 Predicted toxicity risks of designed compounds using "TOPKAT" module

Compound code	Mouse female NTP carcinogen	Mouse male NTP carcinogen	Rat male NTP carcinogen	Ames mutagen	Skin irritancy
4a	-	-	-	-	-
4b	-	-	-	-	-
4c	-	-	-	-	-
4d	-	-	-	-	-
4e	-	-	-	-	-
4f	-	-	-	-	-
4g	-	-	-	-	-
4h	-	-	-	-	-
4i	-	-	-	-	-
4j	-	-	-	-	-

Note: (-) indicates no toxicity risk.

TABLE 3 Docking interactions and binding energy of title compounds **4**(**a-j**) with 1J3K

Compounds code	Docking interaction	(-) Binding energy (kcal mol^{-1})	K_i (mM)
4a	Ser111, Ile112, Arg122	184.31	0.392
4b	Arg59, Arg122	153.10	8.450
4c	Arg122	172.32	0.868
4d	Phe58, Ser111, Ile112, Arg122	224.14	13.840
4e	Phe58, Phe116, Arg122	178.13	123.630
4f	Arg122	160.41	0.205
4g	Arg59, Ser120, Arg122	160.55	7.260
4h	Arg59, Ser120, Arg122	199.05	7.380
4i	Phe58, Phe116, Arg122	184.89	0.673
4j	Ser111, Ile112, Phe116, Arg122	201.72	0.169

obtained by the reaction between 2,4,6-trichloro-1,3,5-triazine (**1**) with aqueous ammonia at 0°C to 5°C for 2 hours. The di-substituted triazine **3**(**a-j**) was achieved by reaction between 4,6-dichloro-1,3,5-triazin-2-amine (**2**) with different aliphatic and aromatic amines (**a-j**) at 27°C to 31°C, 1 bar pressure, 15 W power for 10 minutes in the presence of a base. Whereas, the synthesis of title compounds **4**(**a-j**) was achieved by reacting di-substituted triazine **3**(**a-j**) with excess amount of *p*-aminobenzoic acid (PABA) in 10 mL closed microwave vessel at 100°C, 15 bars pressure and 40 W power for 20 minutes. The synthesized compounds were characterized by different physicochemical properties like solubility, physical state, color, melting point and determination of R_f value by TLC using solvent system of methanol: ethylacetate: chloroform (0.5:0.5:1).

The title compounds were characterized by FT-IR, MS, ^{1}H NMR, ^{13}C NMR spectroscopy. The mass spectrometry showed an ion as M+H for each compound. In the ^{1}H NMR spectra of **4**(**a-j**), singlet between δ 9 and 12 ppm was assignable to the acidic proton at *para* position, and singlet at δ 2.5/4.8 ppm corresponded to the C—NH protons, and the protons in the aromatic ring appeared in the range of δ 6 to 7.8 ppm as characteristic doublet signal. ^{13}C-NMR showed the appearance of carbon signal at about δ 166 to 185 ppm for carboxyl and at about δ 150 to 165 ppm for triazine nucleus. Characteristic FT-IR peaks were observed in the region 3400 to 3200 cm^{-1} due to secondary N—H stretching at 1,3,5-triazine ring, another peak at 3000 to 2500 cm^{-1} (broad) due to OH group present in COOH at phenyl ring and 1600 to 1150 cm^{-1} due to C—C, C—N stretching of 1,3,5-triazine ring. But in some cases, the OH stretching of COOH overlapped on C—H stretching in the region of 3000 to 2400 cm^{-1}.

2.3 | In-vitro antimalarial activity

The concept of hybrid drugs/compounds has revolutionized the area of antimalarial drug discovery. These compounds are designed and developed by adjoining two or different chemical entities with different mechanism of action/pharmacological action via covalent linker into a single molecule. Particularly, in the present study, two different pharmacophores, for instance PABA and 1,3,5-triazine have own antimalarial activity via different mechanisms which results in enhanced activity.[21] The results of In-vitro

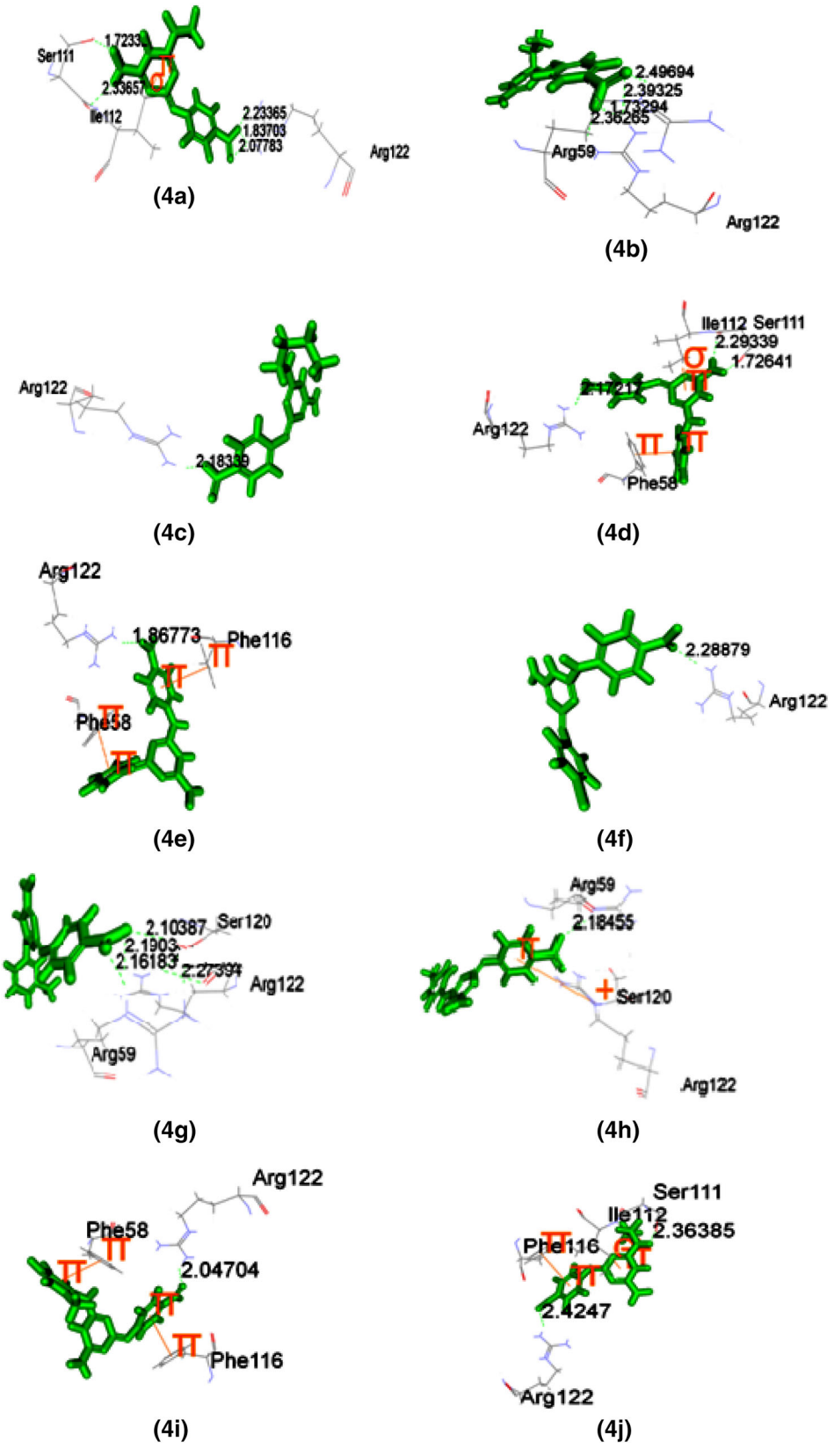
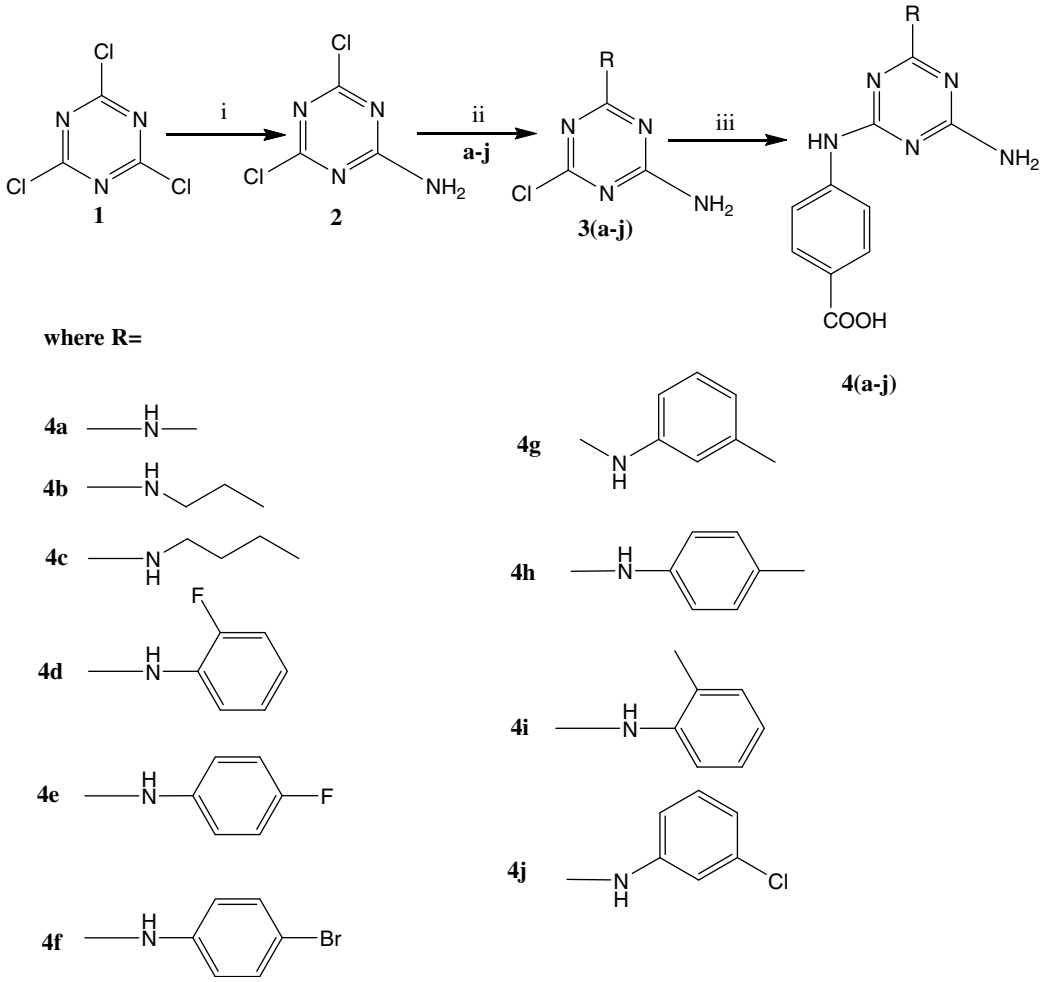


FIGURE 1 Docking poses of *p*-aminobenzoic acid-substituted 1,3,5-triazine derivatives **4**(**a**-**j**) in mutant type *Pf*-DHFR-TS

antimalarial activity of synthesized compounds **4**(**a**-**j**) were identified against chloroquine-sensitive (3D7) strains at 5 μg mL^{-1} using chloroquine as standard and the results are shown in Table 4. All synthesized derivatives **4**(**a**-**j**) showed IC_{50} in the range of 4.46 to 79.72 μg mL^{-1} along with inhibition of parasitemia in terms of % dead rings and trophozoites from 3% to 47% at 5 μg mL^{-1} dose. Compound **4f** having a *para*-bromo showed high antimalarial activity



SCHEME 1 Reagents and conditions: (i) Aq. Ammonia, Diethyl ether, 0°C to 5°C, stirring for 2 hours. (ii) Various amines (**a-j**), 1,4-dioxane, 27°C to 31°C, 1 bar, 15 W, 10 minutes. (iii) PABA, Dimethyl sulfoxide (DMSO), 100°C, 15 bars, 40 W, 20 minutes

with IC_{50} 4.46 μg mL^{-1} along with 47% parasitaemia at 5 μg mL^{-1} dose level. Whereas, the replacement of aromatic with aliphatic amine, for instance compound **4a** and **4b**, the activity was reduced considerably. The butylamino substituted compound **4c** showed good antimalarial activity with IC_{50} 6.69 μg mL^{-1}. The presence of propylamine (**4b**) in place of butylamine (**4c**) showed mild antimalarial activity. The 3-chlorophenyl substituted compound **4j** showed moderate antimalarial activity with IC_{50} 7.09 μg mL^{-1} along with 31% of parasitaemia at 5 μg mL^{-1} dose. Compounds **4g**, **4h** and **4i** containing electron-donating groups, such as *meta*-CH_3, *para*-CH_3, and *ortho* CH_3, respectively, showed mild to moderate antimalarial activity with parasitaemia inhibition 2% to 6.5%. Compounds **4d** and **4e** containing electron-withdrawing groups, such as, *ortho*-fluoro and *para*-fluoro, respectively, also showed mild antimalarial activity with 7% to 10% of parasitaemia at 5 μg mL^{-1} dose level. All compounds were tested for cytotoxicity against MCF 12A (normal epithelial cell) by the MTT assay method and found non-toxic at the highest test dose of 150 μg mL^{-1}.

SAR study indicated that substitution of aliphatic amine such as propyl amine (**4b**) and butyl amine (**4c**) at 1,3,5-triazine appears to influence the antimalarial activity, whereas on replacing aliphatic amine by aromatic amine viz 2-fluoroaniline (**4d**), 4-fluoroaniline (**4e**), *para*-toluidine (**4h**), *meta*-toluidine (**4g**), *ortho*-toluidine (**4i**) showed reduction in the activity. These results were found in agreement with Redley et al[22] which showed that, presence of aliphatic chain on 4-aminoquinoline from carbon chain length from two to six increase the antimalarial activity. The above result indicated that smaller group favors antimalarial activity while bigger groups were detrimental for the activity. This might be due to steric hindrance which interferes with the binding of ligand to the receptor. On the other hand, compounds with electron-withdrawing group (*para*-Br, **4f**) and (*meta*-Cl, **4j**) at phenyl ring of 1,3,5-triazine showed good antimalarial activity. These results were further found in agreement with our earlier study[23] and Pathak et al,[24] where electron withdrawing group enhances antimalarial activity. The

activity showed to be reduced on introduction of electron-donating groups, such as, **4g**, **4h** and **4i** having *meta*-CH_3, *para*-CH_3 and *ortho*-CH_3, respectively. Based on the above observation the SAR could be summarized in Figure 2.

TABLE 4 In-vitro antimalarial evaluation of title compounds **4(a-j)** against chloroquine-sensitive (3D7) strains

Serial No.	Compound code	% Dead rings, trophozoites[a] 3D7 (5 μg mL^{-1} dose)	IC50 (μg mL^{-1})
1	**4a**	3.12 ± 0.16	16.82 ± 0.54
2	**4b**	22.38 ± 0.72	9.89 ± 0.36
3	**4c**	37.24 ± 0.88	6.69 ± 0.28
4	**4d**	7.56 ± 0.20	11.61 ± 0.42
5	**4e**	10.62 ± 0.44	10.06 ± 0.21
6	**4f**	47.48 ± 1.02	4.46 ± 0.18
7	**4g**	2.08 ± 0.09	79.72 ± 1.66
8	**4h**	6.56 ± 0.32	12.98 ± 0.12
9	**4i**	4.06 ± 0.12	14.85 ± 0.48
10	**4j**	31.62 ± 0.22	7.09 ± 0.22
11	Chloroquine (CQ) (0.7 μg mL^{-1})	50.54 ± 0.48	NIL

[a]Data are the means ± SD of n = 3.

3 | EXPERIMENTAL

3.1 | In-silico screening

The in-silico study included design and screening of virtual library, validation of the docking protocol by root mean square deviation (RMSD) calculation and docking of designed compounds at the active binding sites of the target protein. Preliminary screening of the compounds was done using software "Molinspiration cheminformatics"[25] and "Accelrys Discovery Studio version 3.0." Molinspiration cheminformatics provides prediction of different molecular properties (Table 1) while TOPKAT module of Accelrys Discovery Studio version 3.0 predicts toxicity risks of the compounds based on their molecular structure (Table 2). According to Accelrys Discovery Studio tutorial, the three models (Table 2) for determining carcinogenicity on rodents were based on the critical review of technical reports on rodent carcinogenicity conducted by National Cancer Institute and National Toxicology Program (NTP) utilizing hybrid mice and inbred rats.

3.2 | ADME prediction

The in-built ADMET model of Accelrys Discovery Studio version 3.0 for predicting human intestinal absorption of the compounds was developed using descriptors that

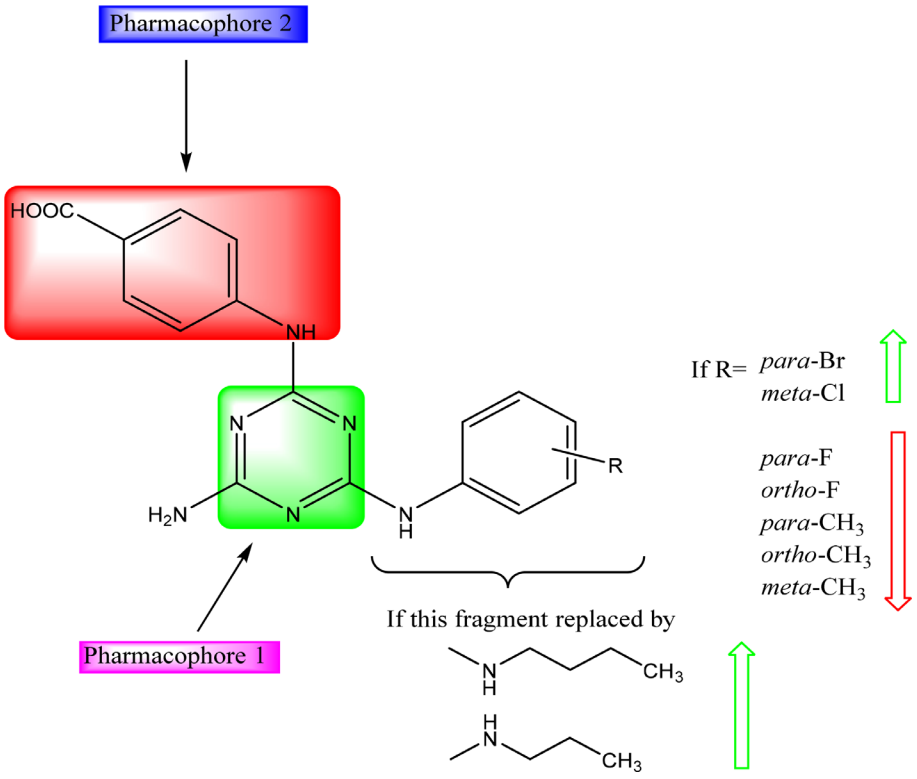


FIGURE 2 Structure–activity relationship of hybrid PABA substituted–1,3,5-triazine derivatives

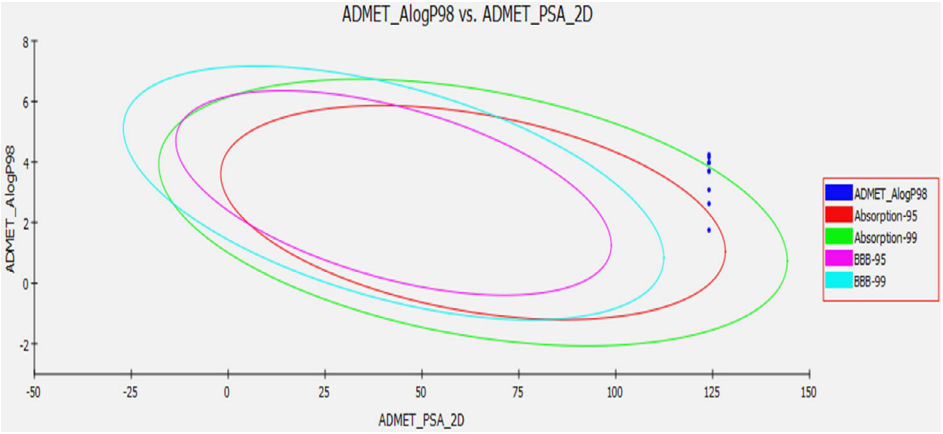


FIGURE 3 Confidence ellipses of human intestinal absorption for selected compounds

include AlogP98 and 2D polar surface area (PSA_2D) which was used in plotting the confidence ellipses. Compounds that were outside 95% and 99% ellipse region (Figure 3) are poorly absorbed compounds (<30% absorbed).[26,27]

3.3 | Molecular docking studies

3.3.1 | Protein preparation

The compounds that were passed through both the preliminary screening filters were docked at the binding sites of the mutant *Pf*-DHFR protein using CDOCKER docking protocol of Accelrys Discovery Studio version 3.0. The crystal structure of quadruple mutant *Pf*-DHFR-TS complex was obtained from RCSB Protein Data Bank as PDB entry code: 1J3K. CDOCKER docking protocol was validated by RMSD calculation, which was less than 2 Å in all cases. Co-crystallized ligand WR99210 and water molecules were removed and cofactor NADPH was allowed to retain. Finally, receptor was prepared according to the requirements of the docking protocol.

3.3.2 | Ligand preparation

The structures of the ligands were drawn in ChemDraw ultra 8.0 and energy minimization was done by applying CHARMm forcefield to create a stable structure.

3.3.3 | Docking

The most stable conformer of ligands was docked into the active site of protein 1J3K, which uses CHARMm based molecular dynamics program.

3.3.4 | Binding energy calculation

The average of the binding energies of the five poses of each complex was calculated.

3.4 | Chemistry

The melting points were determined using melting point instrument M-560: Buchi Switzerland, FT-IR spectra were recorded using Bruker ALPHA ECO-ATR, UV spectra were recorded using UV/Visible Spectrophotometer (Lab India analytical: UV 3200), ^{1}H NMR and ^{13}C NMR spectra were recorded respectively at 400 MHz and 100 MHz in DMSO-d_6 using Bruker Avance II 400 NMR Spectrometer along with TMS as the internal standard where chemical shift (δ) values were given in parts per million (ppm). Molecular mass was recorded on Waters Q-TOF Micromass Spectrometer. Compounds were synthesized using microwave synthesizer (CEM, Discover System Model No. 908010). All commercially available chemicals and reagents with specifications were directly used without further purification.

The general synthetic procedure of the desired compounds **2**, **3**(**a-j**) and **4**(**a-j**) were accomplished by nucleophilic substitution according to the reaction Scheme 1.

3.4.1 | Synthetic procedure for 4,6-dichloro-1,3,5-triazin-2-amine (2)

0.02 mol of 2,4,6-trichloro-1,3,5-triazine (**1**) was dissolved in 30 mL of ice-cold diethyl ether in a round bottom flask followed by addition of equimolar ice-cold aq. ammonia solution (25%). The reaction mixture was stirred for 2 hours in presence of $NaHCO_3$ as a base maintaining at 0°C to 5°C. The product was obtained as (**2**) white needle

shaped crystals (84% yield) with melting point of 229°C to 231°C.

3.4.2 | General procedure for synthesis of disubstituted 1,3,5-triazine derivatives 3 (a-j)

0.002 mol of 4,6-dichloro-1,3,5-triazin-2-amine (**2**) was dissolved in 5 mL of 1,4-dioxane followed by addition of 0.004 mol of various amines (**a-j**) and stirred in closed vessel in microwave synthesizer at 27°C to 31°C, 1 bar pressure, 15 W power for 10 minutes in the presence of $NaHCO_3$ as a base. The reaction mixture was dried and the product was recrystallized with suitable solvent to afford compounds **3**(**a-j**).

3.4.3 | General synthetic procedure for title compounds 4(a-j)

In the final step, corresponding to the synthesis of 2,4,6-trisubstituted-1,3,5-triazine derivatives **4**(**a-j**) using microwave irradiation. To this 0.001 mol of **3**(**a-j**) was dissolved in 2 mL DMSO followed by addition of excess of *p*-aminobenzoic acid (PABA) and was stirred in 10 mL closed microwave vessel at 100°C, 15 bars pressure and 40 W power for 20 minutes. The desired products **4**(**a-j**) were recrystallized.

*4-(4-amino-6-(methylamino)-1,3,5-triazin-2-ylamino) benzoic acid (**4a**)*

Off-white solid; Yield 59%; R_f 0.46; mp 260°C; FTIR (cm^{-1}): 3246 (N—H stretch, secondary), 3100 (OH stretch, COOH, broad), 2937 (C—H stretch), 1590 (N—H bend), 1559, 1494 (C—C stretch, aromatic), 1341, 1241, 1167 (C—N stretch, aromatic); λmax 296 nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.57 (s, 3H, CH_3), 4.65 (s, 2H, 2NH), 4.84 (s, 2H, NH_2), 6.53 to 6.50 (d, J = 12 Hz, 2H, Ar—H), 7.10 to 7.08 (d, J = 8 Hz, 2H, Ar—H), 11.36 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.60, 165.75, 163.51, 152.88, 147.73, 129.90, 114.95, 112.52, 27.26; MS For $C_{11}H_{12}N_6O_2$ $(M+H)^+$: 260.65.

*4-(4-amino-6-(propylamino)-1,3,5-triazin-2-ylamino) benzoic acid (**4b**)*

Off-white solid; Yield 67%; R_f 0.48; mp 154°C; FTIR (cm^{-1}): 3345 (N—H stretch), 3219 (OH stretch, COOH), 2963 (C—H stretch), 1597 (N—H bend), 1554, 1514 (C—C aromatic stretch), 1372 (C—H aromatic stretch), 1242, 1167 (C—N aromatic stretch); λmax 273 nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.65 to 0.62 (t, J = 12 Hz, 3H, CH_2—CH_3), 1.37 to 1.27 (m, 2H, $CH_2CH_2CH_3$), 2.25 (s, 4H, NH), 3.07 to 3.03 (t, J = 16 Hz, 2H, CH_2CH_2), 6.31 to 6.29 (d, J = 8 Hz, 2H, Ar—H), 7.35 to 7.33 (d, J = 8 Hz, 2H, Ar—H), 10.14 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.55, 166.88, 152.76, 131.05, 129.87, 117.06, 112.63, 78.82, 21.95, 20.30, 11.07; MS For $C_{13}H_{16}N_6O_2$ $(M+H)^+$: 289.3.

*4-(4-amino-6-(butylamino)-1,3,5-triazin-2-ylamino) benzoic acid (**4c**)*

Off-white solid; Yield 66%; R_f 0.5; mp 181°C; FTIR (cm^{-1}): 3248 (N—H stretch, secondary), 3091 (OH stretch, COOH), 2951, 2861 (C—H stretch), 1626 (C═C stretch), 1536, 1406 (C—C aromatic stretch), 1358 (C—H aromatic stretch), 1282, 1166 (C—N aromatic stretch); λmax 240 nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.91 to 0.88 (t, J = 12 Hz, 3H, CH_2CH_3), 1.34 to 1.30 (t, J = 16 Hz, 2H, CH_2CH_2), 1.53 to 1.46 (m, 2H, $CH_2CH_2CH_2$), 3.29 to 3.20 (m, 2H, $CH_2CH_2CH_3$), 4.84 (s, 4H, NH), 6.57 to 6.55 (d, J = 8 Hz, 2H, Ar—H), 7.65 to 7.63 (d, J = 8 Hz, 2H, Ar—H), 9.28 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.58, 165.29, 152.89, 131.10, 129.85, 117.04, 112.51, 78.74, 30.81, 19.56, 13.71; MS For $C_{14}H_{18}N_6O_2$ $(M+H)^+$: 302.61.

*4-(4-amino-6-(2-fluorophenylamino)-1,3,5-triazin-2-ylamino)benzoic acid (**4d**)*

Off-white solid; Yield 64%; R_f 0.72; mp 204°C; FTIR (cm^{-1}): 3359 (N—H stretch, secondary), 3065 (OH stretch, COOH), 2933, 2833, 2527 (C—H stretch), 1596, 1505, 1447, 1413 (C—C aromatic stretch), 1247, 1166, 1108 (C—N aromatic stretch); λmax 290 nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.56 (s, 4H, NH), 6.59 to 6.58 (d, J = 4 Hz, 2H, Ar—H), 7.22 to 7.06 (m, 4H, Ar—H), 7.66 to 7.64 (d, J = 8 Hz, 2H, Ar—H), 9.55 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.60, 167.19, 164.80, 164.13, 152.86, 144.23, 131, 129.87, 125.53, 123.78, 118.60, 117.08, 115.14, 112.54; MS For $C_{16}H_{13}FN_6O_2$ $(M+H)^+$: 340.31.

*4-(4-amino-6-(4-fluorophenylamino)-1,3,5-triazin-2-ylamino)benzoic acid (**4e**)*

Green solid; Yield 64%; R_f 0.52; mp 150°C; FTIR (cm^{-1}): 3221 (N—H stretch, secondary), 3086 (OH stretch, COOH), 2864 (C—H stretch), 1599, 1497, 1413 (C—C aromatic stretch), 1315 (C—H aromatic stretch), 1283, 1217, 1168 (C—N aromatic stretch); λmax 230 nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.57 (s, 4H, NH), 6.63 to 6.61 (d, J = 8 Hz, 2H, Ar—H), 7.16 to 7.04 (m, 4H, Ar—H), 7.69 to 7.66 (d, J = 12 Hz, 2H, Ar—H), 9.58 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.53, 167.19, 163.61, 158.95, 151.99, 144.15, 131.08, 129.92, 118.86, 117.76, 114.75, 113.08, 111.09; MS For $C_{16}H_{13}FN_6O_2$ $(M+H)^+$: 340.31.

*4-(4-amino-6-(4-bromophenylamino)-1,3,5-triazin-2-ylamino)benzoic acid (**4f**)*

Off-white solid; Yield 64%; R_f 0.76; mp 277°C; FTIR (cm^{-1}): 3355 (N—H stretch, secondary), 2914 (OH stretch, COOH), 2619 (C—H stretch), 1669 (C═C stretch), 1593, 1562, 1483, 1401 (C—C aromatic stretch), 1309 (C—H aromatic stretch), 1239, 1163, 1110 (C—N aromatic stretch); λmax 233 nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.57 (s, 4H, NH), 6.61 to 6.58 (d, J = 12 Hz, 2H, Ar—H), 7.47 to 7.38 (m, 4H, Ar—H), 7.68 to 7.66 (d, J = 8 Hz, 2H, Ar—H), 11.24 (s, 1H, COOH); ^{13}C NMR (400 MHz, DMSO-d_6) δ: 167.56, 167.13, 163.83, 152.73, 149.85, 139.12, 130.90, 129.94, 122.10, 117.16, 112.60; MS For $C_{16}H_{13}BrN_6O_2$ $(M+H)^+$: 401.19.

*4-(4-(m-toluidino)-6-amino-1,3,5-triazin-2-ylamino) benzoic acid (**4g**)*

Off-white solid; Yield 65%; R_f 0.65; mp 173°C; FTIR (cm^{-1}): 3269 (N—H stretch, secondary), 2921 (OH stretch, COOH), 2853, 2584 (C—H stretch), 1599, 1479, 1414 (C—C aromatic stretch), 1360 (C—H aromatic stretch), 1254, 1170, 1122 (C—N aromatic stretch); λmax (nm) 290; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.34 (s, 3H, CH_3), 2.55 (s, 4H, NH), 6.58 to 6.56 (d, J = 8 Hz, 2H, Ar—H), 6.82 to 6.79 (t, J = 12 Hz, 1H, Ar—H), 7.20 to 7.14 (m, 3H, Ar—H), 7.68 to 7.66 (d, J = 8 Hz, 2H, Ar—H), 9.33 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.58, 163.98, 152.89, 139.66, 139.03, 137.43, 131.12, 128.09, 123.22, 122.69, 117.05, 112.55, 78.77, 21.20; MS For $C_{17}H_{16}N_6O_2$ $(M+H)^+$: 336.35.

*4-(4-(p-toluidino)-6-amino-1,3,5-triazin-2-ylamino) benzoic acid (**4h**)*

Off-white solid; Yield 66%; R_f 0.55; mp 182°C; FTIR (cm^{-1}): 3460, 3360 (N—H stretch), 2827 (OH stretch, COOH, broad) (C—H stretch), 2539, 1657 (C═C stretch), 1589, 1495, 1408 (C—C aromatic stretch), 1279, 1240, 1164 (C—N aromatic stretch); λmax 242 nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.29 (s, 3H, CH_3), 2.56 (s, 4H, NH), 6.60 to 6.58 (d, J = 8 Hz, 1H, Ar—H), 7.09 to 7.07 (d, J = 8 Hz, 2H, Ar—H), 7.68 to 7.65 (d, J = 12 Hz, 2H, Ar—H), 9.27 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.63, 152.60, 136.58, 133.52, 131.12, 128.67, 121.95, 120.75, 117.31, 112.71, 79, 20.47; MS For $C_{17}N_{16}N_6O_2$ $(M+H)^+$: 336.35.

*4-(4-(o-toluidino)-6-amino-1,3,5-triazin-2-ylamino) benzoic acid (**4i**)*

White solid; Yield 62%; R_f 0.55; mp 180°C; FTIR (cm^{-1}): 3455, 3365 (N—H stretch), 2913 (OH stretch, COOH), 2534 (C—H sretch), 1665 (C═C stretch), 1593, 1505, 1411 (C—C aromatic stretch), 1278, 1164, 1118 (C—N aromatic stretch); λmax 295 nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.21 (s, 3H, CH_3), 5.80 (s, 4H, NH), 6.56 to 6.54 (d, J = 8 Hz, 2H, Ar—H), 6.72 to 6.70 (d, J = 8 Hz, 2H, Ar—H), 7.00 to 6.98 (d, J = 8 Hz, 2H, Ar—H), 7.71 to 7.62 (m, 2H, Ar—H), 11.84 (s, 1H, COOH); ^{13}C NMR: (100 MHz, DMSO-d_6) δ: 167.55, 165.01, 153, 137.30, 131.15, 129.91, 125.57, 124.23, 116.94, 112.54, 111.41, 78.83, 18.03; MS For $C_{17}H_{16}N_6O_2$ $(M+H)^+$: 336.35.

*4-(4-amino-6-(3-chlorophenylamino)-1,3,5-triazin-2-ylamino)benzoic acid (**4j**)*

Off-white solid; Yield 64%; R_f 0.55; mp 196°C; FTIR (cm^{-1}): 3354 (N—H stretch), 2890 (OH stretch, COOH), 2617 (C—H stretch), 1632 (C═C stretch), 1596, 1574, 1514, 1420 (C—C aromatic stretch), 1243, 1170, 1109 (C—N aromatic); λmax 248 nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.53 (s, 4H, NH), 6.92 to 6.90 (d, J = 8 Hz, 2H, Ar—H), 7.03 to 7.01 (d, J = 8 Hz, 1H, Ar—H), 7.16 to 7.14 (d, J = 8 Hz, 1H, Ar—H), 7.31 to 7.27 (t, J = 16 Hz, 1H, Ar—H), 7.79 to 7.75 (d, J = 16 Hz, 1H, Ar—H), 7.93 to 7.88 (d, J = 20 Hz, 2H, Ar—H), 9.64 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 167.14, 163.23, 149.83, 147.12, 140.86, 133.05, 130.95, 121.58, 119.87, 118.70, 116.20, 78.79; MS For $C_{16}H_{13}ClN_6O_2$ $(M+H)^+$: 356.77.

3.5 | In-vitro antimalarial activity

In vitro antimalarial screening of synthesized compounds was performed against chloroquine-sensitive *P falciparum*

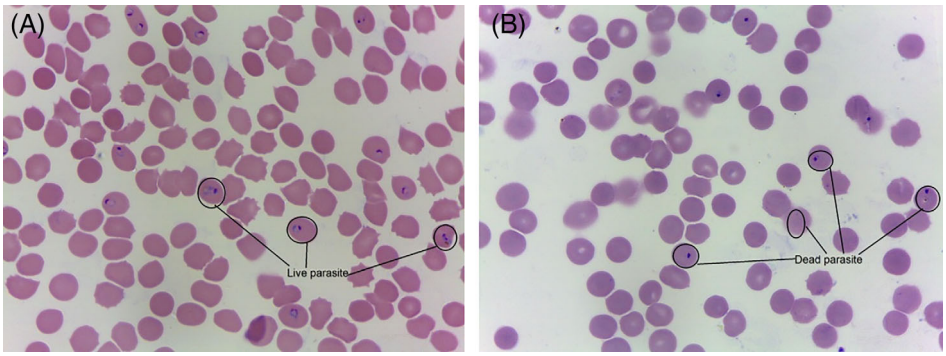


FIGURE 4 A, Negative control (3D7 strain) and B, Drug treated parasite (3D7 strain)

strain (3D7) using Giemsa stained slide method (Light microscopy).

3.6 | Preparation of the parasite

The chloroquine sensitive 3D7 strain of *P falciparum* was routinely maintained in medium, RPMI (Roswell Park Memorial Institute)-1640 supplemented with 25 mmol 4-(2-hydroxyethyl)-1-piperazin-ethane sulphonic acid (HEPES), 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. The initial parasitemia of 0.8% to 1.5% at 3% haematocrit in a total volume of 200 µL of medium RPMI-1640 was uniformly maintained during the assay.

3.7 | In-vitro antimalarial efficacy test

The in-vitro antimalarial assay was carried out according to micro-assay of Rieckmann and co-workers[28] in 96-well microtiter plates, with minor modifications. A stock solution of 5 mg mL^{-1} of each of the test samples was prepared in DMSO and subsequently diluted with culture medium. The test compounds in 20 µL volume at 50 µg mL^{-1} concentration in a duplicate well were incubated with parasitized cell preparation at 37°C and 5% CO_2 level in a carbon dioxide candle incubator. 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 (Figure 4) was determined by counting a total of 100 asexual parasites microscopically using chloroquine as reference drugs.

4 | CONCLUSION

In summary, hybrid *p*-aminobenzoic acid-substituted 1,3,5-triazine derivatives were synthesized and antimalarial screening results revealed compound **4f** and **4c** as promising antimalarial candidate. However, further studies are warranted to elucidate the mechanism of action of reported derivatives as antimalarial agents.

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REFERENCES

[1] WHO. **2018**. 2018. http://www.who.int/malaria/publications/world-malaria-report-2017/report/en/
[2] R. Lozano, N. Fullman, D. Abate, et al., *Lancet* **2018**, *392*, 2091.
[3] M. Sergio, P. Davide, C. Paolo, B. Nicoletta, M. Diego, *ChemMedChem* **2008**, *3*, 873.
[4] S. K. Mekonnen, A. Aseffa, N. Berhe, T. Teklehaymanot, R. M. Clouse, T. Gebru, G. Medhin, T. P. Velavan, *Malar. J.* **2014**, *13*, 244.
[5] G. C. Cerqueira, I. H. Cheeseman, S. F. Schaffner, S. Nair, M. McDew-White, A. P. Phyo, et al., *Genome Biol.* **2017**, *18*, 78.
[6] T. M. Lopera-Mesa, A. Kushwaha, A. Mohmmed, V. S. Chauhan, *Vaccine* **2008**, *26*, 1335.
[7] C. Stella, P. Barbara, S. Virginia, C. Anna, M. Alessandra, B. Paola, et al., *Eur. J. Med. Chem.* **2017**, *142*, 523.
[8] U. P. Singh, H. R. Bhat, P. Gahtori, *J. Mycol. Med.* **2012**, *22*(2), 134.
[9] B. Singh, H. R. Bhat, M. K. Kumawat, U. P. Singh, *Bioorg. Med. Chem. Lett.* **2014**, *24*(15), 3321.
[10] V. Dubey, M. Pathak, H. R. Bhat, U. P. Singh, *Chem. Biol. Drug Des.* **2012**, *80*(4), 598.
[11] N. Sakakibara, G. Balboni, C. Congiu, V. Onnis, Y. Demizu, T. Misawa, M. Kurihara, Y. Kato, T. Maruyama, M. Toyama, M. Okamoto, M. Baba, *Antivir. Chem. Chemother.* **2015**, *24*(2), 62.
[12] J. K. Srivastava, G. G. Pillai, H. R. Bhat, A. Verma, U. P. Singh, *Sci. Rep.* **2017**, *7*(1), 5851.
[13] M. O. Sarhan, M. A. Motaleb, I. T. Ibrahim, M. M. Anwar, W. A. Zaghary, *Mini Rev. Med. Chem.* **2018**, *18*(15), 1302.
[14] P. Gahtori, S. K. Ghosh, P. Parida, A. Prakash, K. Gogoi, H. R. Bhat, U. P. Singh, *Exp. Parasitol.* **2012**, *130*, 292.
[15] D. Indorkar, A. Parteti, O. P. Chourasia, S. N. Limaye, *Int. J. Curr. Microbiol. App. Sci.* **2013**, *2*, 283.
[16] A. Nzila, A. W. Steve, K. Marsh, F. G. S. Paul, E. John, *Trend Parasitol.* **2005**, *21*, 292.
[17] R. Li, R. Sirawaraporn, P. Chitnumsub, W. Sirawaraporn, J. Wooden, F. Athappilly, S. Turley, W. G. J. J. Hol, *Mol. Biol.* **2000**, *295*, 307.
[18] W. K. Milhous, N. F. Weatherly, J. H. Bowdre, R. E. Desjardins, *Antimicrob. Agents Chemother.* **1985**, *27*, 525.
[19] B. Meunier, *Acc. Chem. Res.* **2008**, *41*(1), 69.
[20] Y. Yuthavong, B. Tarnchompoo, T. Vilaivan, P. Chitnumsub, S. Kamchonwongpaisan, S. A. Charman, D. N. McLennan, K. L. White, L. Vivas, E. Bongard, C. Thongphanchang, S. Taweechai, J. Vanichtanankul, R. Rattanajak, U. Arwon, P. Fantauzzi, J. Yuvaniyama, W. N. Charman, D. Matthews, *Proc. Natl. Acad. Sci. USA* **2012**, *109*(42), 16823-16828.
[21] D. Agarwal, R. D. Gupta, S. K. Awasthi, *Antimicrob. Agents Chemother.* **2017**, *61*(5), e00249-17.
[22] R. G. Ridley, H. Hofheinz, H. Matile, C. Jaquet, A. Dorn, R. Masciadri, et al., *J. Antimicrob. Chemother* **1996**, *40*, 1846.
[23] H. R. Bhat, U. P. Singh, P. S. Yadav, V. Kumar, P. Gahtori, A. Das, et al., *Arab. J. Chem.* **2016**, *9*(1), S625-S631.
[24] M. Pathak, H. Ojha, A. K. Tiwari, D. Sharma, M. Saini, R. Kakkar, *Chem. Cent. J.* **2017**, *11*(1), 132.
[25] http://www.molinspiration.com/cgi-bin/properties.
[26] W. J. Egan, K. M. Merz, J. J. Baldwin, *J. Med. Chem.* **2000**, *43*, 3867.

[27] G. Lauri, W. J. Egan, *Adv. Drug Deliv. Rev.* **2002**, *54*, 273.
[28] K. H. Rieckmann, G. H. Campbell, L. J. Sax, J. E. Mrem, *Lancet* **1978**, *1*, 22.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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