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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of novel 4,6-diaryl-2-aminopyrimidines as potential antiplasmodial agents

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Abstract A novel series of 4,6-diaryl-2-aminopyrimidines **8a–o** has been synthesized and evaluated for in vitro antiplasmodial activity against *Plasmodium falciparum*. Out of the 15 compounds synthesized and tested, 6 compounds have shown IC_{50} values in the range of 1.61–9.53 μg/mL. These compounds are several times more potent than chloroquine and quinine, the two standard drugs used for the purpose of comparison.

Keywords 2-Aminopyrimidines · Chloroquine · *Plasmodium falciparum* · Antiplasmodial agents

Introduction

Malaria is re-emerging as the biggest infectious killer with an estimated 3.3 billion people at risk in 2010 and is currently the first priority tropical disease of the World Health Organization (WHO) (World Malaria Report, 2011). The four identified species of the parasite responsible for inflicting human malaria are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale,* and *Plasmodium malariae*. Of these, the former two account for more than 95 % of malaria cases in the world (Snow *et al*., 2005). Although, the disease can be treated in just 48 h, it can cause fatal complications, if the diagnosis and treatment are delayed.

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Pyrimethamine **1**, trimethoprim **2**, and other antifolates (proguanil **3** and chlorproguanil **4**) used for the treatment of *P. falciparum* infection, targeting dihydrofolate reductase (DHFR) (Fig. 1) were identified as potent antimalarial agents several decades ago and are still the mainstay antifolate drugs used in the treatment and prevention of malaria. All these drugs have a higher affinity for binding to *P. falciparum* DHFR over human DHFR. It is well accepted that differences in their binding affinity account for their higher therapeutic index (Rastelli *et al*., 2000). The parasite is observed to develop resistance to conventional antimalarial drugs due to mutations in the active site of their DHFR (Gregson and Plowe, 2005). As a result, discovery of novel antimalarial agents effective against resistant strains of the parasite is one of the greatest challenges facing the control of malaria today (Hyde, 2007; Tripathi *et al*., 2005). Discovering new drugs that attack key targets which are involved in the metabolism of the malarial pathogen is the need of the hour (Wells, 2010; Ridley, 2002; Mui *et al*., 2008).

The structural requirements for DHFR inhibitors have been well documented (Sirichaiwat *et al*., 2004; Warhurst, 1998; Yuthavong, 2002; Mishra *et al*., 2006). The inhibitors should have the following basic characteristics: (a) A heterocyclic ring system which can be accommodated in the hydrophobic active site pocket of DHFR; (b) A suitable group at position 2 of the heterocyclic ring for hydrogen bonding with both the carboxyl oxygens of Asp-54; (c) A substituent at position 4 which can form hydrogen bonding with oxygens of amino acid backbone residues i.e., Ile-164 and Ile-14; (d) Substituent at position 5 should be non-polar as it would be positioned in the hydrophobic core of the enzyme active site. The DHFR-2,4-diamino-pyrimidine interactions (Falco *et al*., 1951; Rollo, 1975) are self explanatory for the development of such moieties

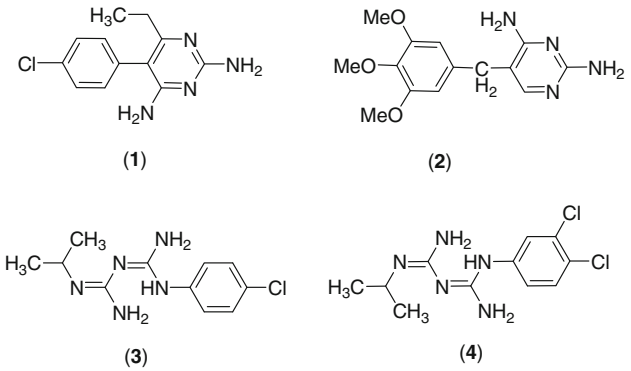


Fig. 1 Clinically used antimalarial agents

(Agarwal *et al*., 2005a, b, c) as antimalarial/antiplasmodial agents. Position-6 of the heterocyclic rings (six-membered) are unexplored. So, it was envisaged to explore the potential of 4,6-diaryl-2-aminopyrimidines as antiplasmodial agents.

In our ongoing program involving the synthesis of small bioactive heterocyclic molecules, we have previously reported antileukemic, antiproliferative and antiplatelet activity of some diaryldiazepines (Ramajayam *et al*., 2008) and diarylpyrimidines (Ramajayam *et al*., 2005; Giridhar *et al*., 2012). This communication describes the synthesis, docking studies and preliminary in vitro evaluation of some novel 4,6-diaryl-2-aminopyrimidines as potential antiplasmodial leads.

Results and discussion

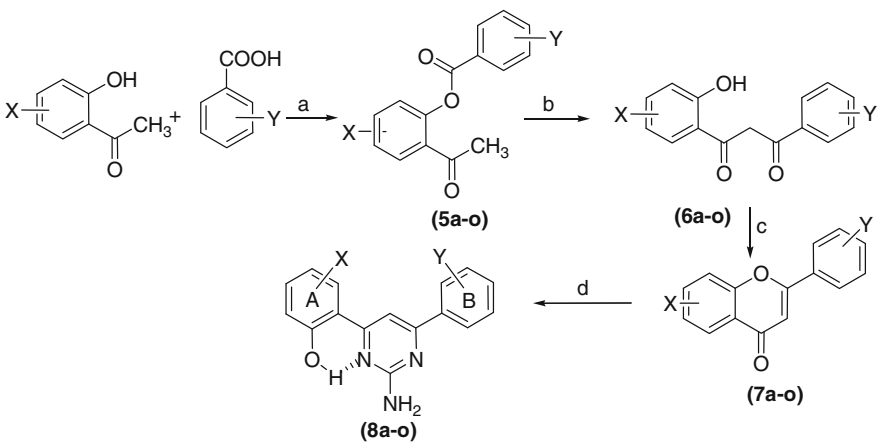
Chemistry

The diarylpyrimidine derivatives reported in this paper were prepared according to Scheme 1. The key intermediate 1,3-diketones **6a**–**o** required for the work were obtained from the esters **5a**–**o**, by base-catalyzed Baker-Venkataraman transformation (Baker, 1933). Condensation of 2-hydroxyacetophenones with various substituted benzoic acids in dry pyridine and $POCl_3$ furnished the esters **5a**–**o**. IR spectra of 1,3-diketones showed absorption bands for C=O in the range of 1,615–1,625 cm^{-1}. Treatment of the diketones with conc. H_2SO_4 led to formation of flavones (**7a**–**o**) (Marder *et al*., 1998). Formation of the flavones **7a**–**o** was confirmed by the appearance of C=O absorption bands at 1,640–1,660 cm^{-1} in their IR spectra. Reaction of the flavones **7a**–**o** with a slight excess of guanidine hydrochloride in alkaline medium afforded 4,6-diaryl-2-amino-pyrimidines **8a**–**o**. All the synthesized compounds were characterized by spectral and elemental analysis.

Compounds **8a**–**o** showed a characteristic peak at around 3,200 cm^{-1} for O–H stretching and asymmetric and symmetric N–H stretching bands at 3,500 and 3,350 cm^{-1}, respectively in their IR spectra. In the ^1H NMR spectra, NH_2 protons appeared at δ 5.1–6.0 and the pyrimidinyl proton (C_5–H) appeared as a sharp singlet at δ 7.3–7.5. A downfield value of δ 13–14 for the –OH protons is indicative of the existence of a partial six-membered ring as shown in the structure. In the ^{13}C NMR spectra, the δ values of the carbons are in confirmity with the structures of the synthesized compounds.

Antiplasmodial activity

All the synthesized compounds were screened for antiplasmodial activity on chloroquine sensitive *P. falciparum* MRC 20 strains. Strain of *P. falciparum* was cultured continuously according to the candle jar method (Trager and Jensen, 1976; Soni and Gupta, 2009), in vitro in human red blood cells (blood type B^+) with 5 % hematocrit in LIQUID RPMI 1640 medium (HIMEDIA) supplemented



Reagents and conditions: (a) $POCl_3$, dry pyridine, rt, 2 h; (b) fused KOH, dry pyridine, rt, 2 h; (c) AcOH, catalytic H_2SO_4, 100 °C, 1 h; (d) Guanidine hydrochloride, KOH, MeOH, reflux, 6-8 h.

Scheme 1 Synthesis of 4,6-diaryl-2-aminopyrimidines

with 25 mM HEPES (Sigma), 0.2 % sodium bicarbonate (Sigma) and 10 % human B^+ serum. Slides of culture were observed after 3, 6, and 24 h for regular development of parasite stages. The inhibition of parasite growth in the drug-treated groups was calculated as follows:

$$\text{Percentage inhibition} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

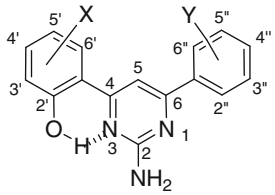
where; "control" is the parasitemia in the non-treated group and "treated" is the parasitemia in the drug-treated group, expressed as percentages. All values are expressed as percentage growth inhibition. Dose–response curves were obtained by plotting percentage inhibition against log concentration. The values of the compounds provided a mid-point value where parasite growth would be 50 %. Linear regression analysis was applied to the linear portion of the sigmoidal curve and IC_{50} values were derived for each test compound. The IC_{50} value of chloroquine and quinine was determined to be 10 and 29 μg/mL, respectively (Karle *et al*., 1992). The IC_{50} values of test compounds are given in Table 1.

Docking studies

The molecular docking studies of 4,6-diaryl-2-aminopyrimidines with the pfDHFR binding site showed very clear preference to the inhibitor binding pocket. Most of the compounds bind in a similar fashion with the 2-aminopyrimidine ring occupying the interior of the deep cleft and its 4-phenyl substitution extending toward the hydrophobic binding cavity. The 2-aminopyrimidine core of the compounds was fit in the pocket by a combination of both van Der Waals and hydrophobic interactions with the protein. The aromatic ring of Phe-58 stabilizes the 4-phenyl ring with bulky benzyloxy substitution by π–π interactions. The hydroxyl group of 4-phenyl group further stabilizes the protein–ligand complex by hydrogen bonding with Ile-164 with distance 2.055 Å in case of benzyloxy and an opposite orientation has been observed in case of 4′-methoxyphenyl showing hydrogen bonding with Gly-166 with distance 1.966 Å.

The substituents at position 4 of the 2-aminopyrimidine ring have significant effect on the inhibitory activity. As per our docking studies, bulkier substituents are preferred at this position due to hydrophobic environment provided by the side chains of Ile-14, Leu-46, Try-48, Ile-164, and Leu-119. Compounds bearing 4′-methoxy and 4′-benzyloxy substituents displayed a binding mode as shown by representative compounds **8b** and **8k** in Figs. 2 and 3, respectively. These compounds showed hydrophobic interactions with Ile-14, Leu-46, Try-48, Ile-164, and Leu-119. In addition to these hydrophobic interactions, π interactions are also found to occur between the π system of Phe-58 and the 4-phenyl ring. The displacement of this benzyloxy moiety with methoxy at 4′- or 5′-position in compounds **8a–h**, led to comparable decrease in the inhibitory activity whereas replacement with halo-substituted phenyl ring at position 6 in compounds **8a**, **8c**, **8f–h**, **8l**, and **8m** cause decrease in the hydrophobic interactions, thus lowering down the inhibition. In contrast, the compounds **8b** and **8i–k** bearing 4′- or 3′-methoxyphenyl ring show similar activity profiles displaying better antiplasmodial effect. However, docking studies that were performed could not explain the reduction in activity of compound **8e** with 3″-methoxy substituent which may be due to steric hindrance because of 5′-methoxy group at position 4.

Table 1 Structures and antiplasmodial activity of the compounds **8a–o**



8a-o

Comp. no.	X	Y	IC_{50}^{a} (μg/mL)[b]
8a	4′-OCH_3	4″-Cl	21.35 ± 3.16[c]
8b	4′-OCH_3	4″-OCH_3	1.78 ± 0.58[d]
8c	4′-OCH_3	4″-F	15.25 ± 2.01[e]
8d	4′-OCH_3	4″-CH_3	8.71 ± 1.37[c]
8e	5′-OCH_3	3″-OCH_3	>25 ± 2.30[e]
8f	5′-OCH_3	2″-Cl	29.35 ± 2.68[e]
8g	5′-OCH_3	3″-Cl	9.53 ± 1.39[c]
8h	5′-OCH_3	4″-F	25.52 ± 2.22[e]
8i	4′-Benzyloxy	4″-OCH_3	5.01 ± 0.98[c]
8j	4′-Benzyloxy	3″-OCH_3	7.61 ± 2.37[c]
8k	4′-Benzyloxy	3″,4″-di-OCH_3	1.61 ± 0.40[d]
8l	4′-Benzyloxy	4″-F	16.67 ± 1.83[e]
8m	4′-Benzyloxy	2″-Cl	18.87 ± 1.88[c]
8n	4′-Benzyloxy	4″-CH_3	14.87 ± 1.62[c]
8o	4′-Benzyloxy	3″-CH_3	35.56 ± 4.10[c]
Chloroquine	–	–	10 ± 2.73[c]
Quinine	–	–	29 ± 2.40[c]

The parasite strain used for the assay is chloroquine sensitive strain of *P. falciparum* MRC 20 and the results are represented as a single reading; average was calculated by counting three different fields of culture slide

[a] The assay is stage specific inhibition assay to check development of schizonts to trophozoites

[b] IC_{50} values ± SD

Cell lysis observed after [c] >25, [d] >12.5, [e] >50 (μg/mL)

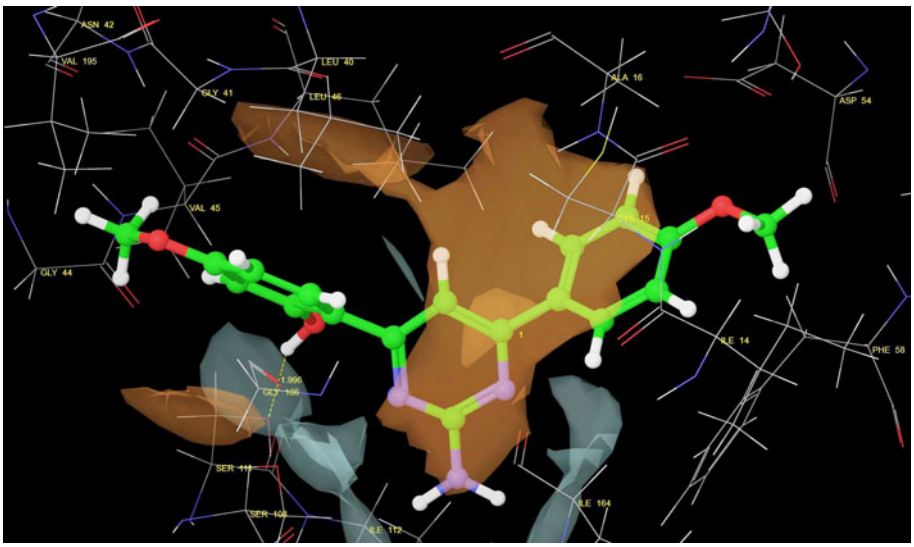


Fig. 2 Docked conformation of compound **8b** in the pfDHFR active site

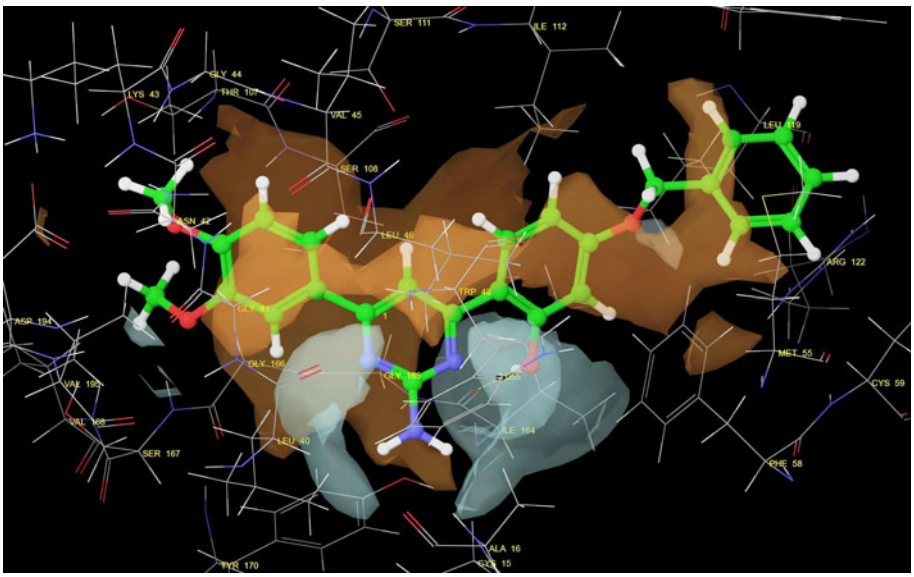


Fig. 3 Docked conformation of compound **8k** in the pfDHFR active site

Structure–activity relationships

The in vitro biological activity of the synthesized pyrimidine derivatives has shown encouraging results against the chloroquine sensitive strain. Out of the 15 novel compounds; two compounds **8b** and **8k** have been found to be more potent than the standard drug chloroquine with IC_{50} values of 1.78 and 1.61 μg/mL, respectively. Compound **8b** bears 4-methoxy substitution on both the phenyl rings of the pyrimidine ring system. Replacement of 4″-methoxy group of phenyl ring of compound **8b** by methyl group caused a decrease in potency (**8d**). The weak activity of halo-substituted compounds might be due to decrease in electron density in the phenyl ring. Compound **8k** with 3″,4″-dimethoxy group on phenyl ring and highly hydrophobic benzyloxy substitution on hydroxyphenyl ring showed the highest potency. Compounds with benzyloxy group and methoxy or methyl groups showed comparable activities. Compounds **8d**, **8g**, **8i**, and **8j** showed IC_{50} values <10 μg/mL.

Conclusion

Fifteen novel 4,6-diaryl-2-aminopyrimidines **8a–o** have been synthesized as potential antiplasmodial drugs. Of the synthesized compounds, five compounds showed IC_{50} values in the range of 1.61–9.53 μg/mL, whereas four compounds showed IC_{50} values comparable to quinine, which was used as a standard drug in the study. These

compounds may serve to be ideal leads for further optimization of their structures to provide newer and safer antimalarial/antiplasmodial drugs.

Experimental

Docking studies

Docking studies were performed with crystallographic 3D structure of pfDHFR receptor (PDB Code: 3QGT) (Vanichtanankul *et al*., 2011) using Glide (Schrödinger, New York, USA, 2009). It performs grid-based ligand docking with energetics and searches for favorable interactions between one or more small ligand molecules and a larger receptor molecule, usually a protein. Docking calculations were first performed in SP mode and then in XP mode. All the molecules were built within Maestro using the Built module and an exhaustive conformational search was carried out for all molecules using OPLS_2005 force field, imposing a cutoff of allowed value of the total conformational energy compared to the lowest-energy state. A minimization cycle for conjugate gradient and steepest descent minimizations were used with default value 0.05 Å for the initial step size and 1.00 Å for the maximum step size.

Chemical studies

Melting points were determined in open capillaries using Toshniwal melting point apparatus and are uncorrected. IR spectra (in cm^{-1}) were recorded using KBr pellets on a Shimadzu 8300 instrument; and ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker Avance II spectrometer (400 MHz), using tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ, in ppm) where s, br, t, and m indicate singlet, broad, triplet, and multiplet, respectively. Elemental analyses were recorded on a Perkin Elmer PE 2400 CHNS analyzer. Mass spectra were recorded on APISciEX mass spectrometer equipped with an electrospray ionization (ESI) interface. Column chromatography was carried out using silica gel (100–200 mesh). Thin-layer chromatography (TLC) was performed on precoated Silica gel Merck plates. Compounds were visualized by illuminating with UV light (254 nm) or exposure to iodine vapors. Solvents were purified using standard purification techniques.

*General method for the preparation of 4,6-diaryl-2-aminopyrimidine derivatives (8**a–o**)*

A mixture of an appropriate flavone **7a–o** (0.5 g, 0.002 mol), guanidine hydrochloride (0.7 g, 0.07 mol) and potassium hydroxide (1.5 g) was refluxed in methanol (30 ml) for 6–8 h. After the completion of the reaction, the mixture was poured into crushed ice-acetic acid mixture (50 g, 30 %). The yellow solid so obtained was filtered, washed with water, and recrystallized from methanol.

*4-(2′-Hydroxy-4′-methoxyphenyl)-6-(4″-chlorophenyl)-2-aminopyrimidine (**8a**)* Yield 33 %; Yellow crystals from MeOH; mp 215–217 °C; IR (KBr) νmax: 3494, 3365, 2923, 1618 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.89 (s, 3H, OCH_3), 5.19 (s, 2H, NH_2), 6.59 (s, 1H, ArH), 7.29–7.30 (m, 2H, ArH), 7.32–7.34 (d, $J = 7.6$, 2H, ArH), 7.40 (1H, Pyri), 7.42–7.44 (d, $J = 7.4$, 2H, ArH); MS *m*/*z*; 328.76 (M+1); Anal. Calcd for $C_{17}H_{14}ClN_3O_2$: C, 62.30; H, 4.31; N, 12.82. Found: C, 62.35; H, 4.22; N, 13.02.

*4-(2′-Hydroxy-4′-methoxyphenyl)-6-(4″-methoxyphenyl)-2-aminopyrimidine (**8b**)* Yield 35 %; Yellow crystals from MeOH; mp 223–225 °C; IR (KBr) νmax: 3410, 3300 (NH_2), 3173.9 (OH), 1635 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.88 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 5.15 (s, 2H, NH_2), 6.54–6.57 (m, 2H, ArH), 6.60 (s, 1H, ArH), 7.35 (s, 1H, pyri), 7.36–7.37 (d, $J = 7.8$, 2H, ArH), 7.42–7.43 (d, $J = 7.84$, 2H, ArH); MS *m*/*z*; 324.13 (M+1); Anal. Calcd for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00. Found: C, 66.78; H, 5.21, N, 13.00.

*4-(2′-Hydroxy-4′-methoxyphenyl)-6-(4″-fluorophenyl)-2-aminopyrimidine (**8c**)* Yield 25 %; Yellow crystals from MeOH; mp 205–207 °C; IR (KBr) νmax: 3500, 3180, 3120, 1620 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.8 (s, 3H, OCH_3), 5.1 (2H, NH_2), 7.39 (s, 1H, Pyri), 6.9–7.6 (7H, ArH), 13.19 (br, 1H, OH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 55.44, 100.91, 101.90, 107.47, 110.56, 115.78, 116.00, 128.35, 129.20, 129.28, 133.25, 160.22, 163.08, 163.28, 163.80, 164.47, 165.87; MS *m*/*z*; 312.1 (M+1); Anal. Calcd for $C_{17}H_{14}FN_3O_2$: C, 65.59; H, 4.53; N, 13.50. Found: C, 65.50; H, 4.62; N, 13.39.

*4-(2′-Hydroxy-4′-methoxyphenyl)-6-(4″-methylphenyl)-2-aminopyrimidine (**8d**)* Yield 16 %; Yellow crystals from MeOH; mp 195–197 °C; IR (KBr) νmax: 3500, 3380, 3120, 1620 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.44 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 5.1 (2H, NH_2), 6.4–8.0 (m, 7H, ArH), 7.34 (s, 1H, Pyri), 13.96 (br, 1H, OH); MS *m*/*z*; 308.2 (M+1); Anal. Calcd for $C_{18}H_{17}N_3O_2$: C, 70.34; H, 5.58; N, 13.67. Found: C, 70.30; H, 5.62; N, 13.60.

*4-(2′-Hydroxy-5′-methoxyphenyl)-6-(3″-methoxyphenyl)-2-aminopyrimidine (**8e**)* Yield 54 %; Yellow crystals from MeOH; mp 158–160 °C; IR (KBr) νmax: 3430, 3320, 3220, 1599 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.8

(s, 3H, OCH_3), 4.1 (s, 3H, OCH_3) 5.1 (2H, NH_2), 7.36 (s, 1H, Pyri), 6.9–7.8 (7H, ArH), 13.19 (br, 1H, OH); MS *m/z*; 324.2 (M+1); Anal. Calcd for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00. Found: C, 66.80; H, 5.36; N, 12.90.

*4-(2′-Hydroxy-5′-methoxyphenyl)-6-(2″-chlorophenyl)-2-aminopyrimidine (**8f**)* Yield 52 %; Yellow crystals from MeOH; mp 163–165 °C; IR (KBr) νmax: 3480, 3310, 3110, 1655 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.8 (s, 3H, OCH_3), 5.2 (2H, NH_2), 7.39 (s, 1H, Pyri), 6.9–7.9 (7H, ArH), 13.09 (br, 1H, OH); MS *m/z*; 328.2 (M+1); Anal. Calcd for $C_{17}H_{14}ClN_3O_2$: C, 62.30; H, 4.31; N, 12.82. Found: C, 62.34; H, 4.27; N, 12.79.

*4-(2′-Hydroxy-5′-methoxyphenyl)-6-(3″-chlorophenyl)-2-aminopyrimidine (**8g**)* Yield 70 %; Yellow crystals from MeOH; mp 178–179 °C; IR (KBr) νmax: 3456, 3352, 3110, 1640 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.8 (s, 3H, OCH_3), 5.2 (2H, NH_2), 7.37 (s, 1H, Pyri), 7.0–7.8 (7H, ArH), 13.84 (br, 1H, OH); ^{13}C NMR (400 MHz, $CDCl_3$): δ 56.13, 102.10, 111.48, 117.36, 119.37, 119.84, 125.27, 127.38, 130.08, 130.78, 135.01, 139.14, 152.24, 154.94, 160.79, 165.16, 166.00; MS *m/z*; 328.2 (M+1); Anal. Calcd for $C_{17}H_{14}ClN_3O_2$: C, 62.30; H, 4.31; N, 12.82. Found: C, 62.36; H, 4.30; N, 12.85.

*4-(2′-Hydroxy-5′-methoxyphenyl)-6-(4″-fluorophenyl)-2-aminopyrimidine (**8h**)* Yield 56 %; Yellow crystals from MeOH; mp 202–203 °C; IR (KBr) νmax: 3460, 3310, 3222, 1640 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.8 (s, 3H, OCH_3), 5.1 (2H, NH_2), 7.34 (s, 1H, Pyri), 6.9–7.8 (7H, ArH), 13.16 (br, 1H, OH); MS *m/z*; 312.2 (M+1); Anal. Calcd for $C_{17}H_{14}FN_3O_2$: C, 65.59; H, 4.53; N, 13.50. Found: C, 65.51; H, 4.57; N, 13.54.

*4-(4′-Benzyloxyphenyl-2′-hydroxy)-6-(4″-methoxyphenyl)-2-aminopyrimidine (**8i**)* Yield 55 %; Yellow crystals from MeOH; mp 226–227 °C; IR (KBr) νmax: 3495, 3347, 3223, 1652 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.4 (s, 3H, OCH_3), 5.12 (s, 2H, OCH_2Ar), 6.5 (2H, NH_2), 6.6–8.1 (m, 12H, ArH), 7.35 (s, 1H, Pyri), 10.07 (br, 1H, OH); MS *m/z*; 400.2 (M+1); Anal. Calcd for $C_{24}H_{21}N_3O_3$: C, 72.16; H, 5.30; N, 10.52. Found: C, 72.21; H, 5.28; N, 10.52.

*4-(4′-Benzyloxyphenyl-2′-hydroxy)-6-(3″-methoxyphenyl)-2-aminopyrimidine (**8j**)* Yield 47 %; Yellow crystals from MeOH; mp 154–155 °C; IR (KBr) νmax: 3400, 3338, 3223, 1652 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.9 (s, 3H, OCH_3), 5.12 (s, 2H, OCH_2Ar), 5.3 (2H, NH_2), 6.6–8.1 (m, 12H, ArH), 7.39 (s, 1H, Pyri), 10.53 (br, 1H, OH); MS *m/z*; 400.2 (M+1); Anal. Calcd for $C_{24}H_{21}N_3O_3$: C, 72.16; H, 5.30; N, 10.52. Found: C, 72.18; H, 5.29; N, 10.52.

*4-(4′-Benzyloxyphenyl-2′-hydroxy)-6-(3″,4″-dimethoxyphenyl)-2-aminopyrimidine (**8k**)* Yield 32 %; Yellow crystals from MeOH; mp 198–200 °C; IR (KBr) νmax: 3420, 3322, 3210, 1647 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.9 (s, 3H, OCH_3), 4.0 (s, 3H, OCH_3), 5.12 (s, 2H, OCH_2Ar), 5.3 (2H, NH_2), 6.9–7.9 (m, 12H, ArH), 7.36 (s, 1H, Pyri), 11.03 (br, 1H, OH); MS *m/z*; 430.2 (M+1); Anal. Calcd for $C_{25}H_{23}N_3O_4$: C, 69.92; H, 5.40; N, 9.78. Found: C, 69.95; H, 5.37; N, 9.77.

*4-(4′-Benzyloxyphenyl-2′-hydroxy)-6-(4″-fluorophenyl)-2-aminopyrimidine (**8l**)* Yield 45 %; Yellow crystals from MeOH; mp 195–196 °C; IR (KBr) νmax: 3493, 3318, 3220, 1652 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 5.12 (s, 2H, OCH_2Ar), 6.5 (2H, NH_2), 6.6–8.1 (m, 12H, ArH), 7.35 (s, 1H, Pyri), 12.01 (br, 1H, OH); MS *m/z*; 388.2 (M+1); Anal. Calcd for $C_{23}H_{18}FN_3O_2$: C, 71.31; H, 4.68; N, 10.85. Found: C, 71.27; H, 4.70; N, 10.86.

*4-(4′-Benzyloxyphenyl-2′-hydroxy)-6-(2″-chlorophenyl)-2-aminopyrimidine (**8m**)* Yield 35 %; Yellow crystals from MeOH; mp 161–162 °C; IR (KBr) νmax: 3450, 3315, 3220, 1652 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 5.10 (s, 2H, OCH_2Ar), 5.15 (2H, NH_2), 7.34–7.39 (m, 5H, ArH), 6.55–7.71 (m, 7H, ArH), 7.35 (s, 1H, Pyri), 13.88 (br, 1H, OH); MS *m/z*; 404.1 (M+1); Anal. Calcd for $C_{23}H_{18}ClN_3O_2$: C, 68.40; H, 4.49; N, 10.40. Found: C, 68.40; H, 4.51; N, 10.40.

*4-(4′-Benzyloxyphenyl-2′-hydroxy)-6-(4″-methylphenyl)-2-aminopyrimidine (**8n**)* Yield 56 %; Yellow crystals from MeOH; mp 215–216 °C; IR (KBr) νmax: 3428, 3323, 3204, 1647 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.4 (s, 3H, CH_3), 5.10 (s, 2H, OCH_2Ar), 6.44 (2H, NH_2), 7.2–7.4 (m, 5H, ArH), 7.4–7.8 (m, 7H, ArH), 7.35 (s, 1H, Pyri); MS *m/z*; 384.1 (M+1); Anal. Calcd for $C_{24}H_{21}N_3O_2$: C, 75.18; H, 5.52; N, 10.96. Found: C, 75.30; H, 5.50; N, 10.90.

*4-(4′-Benzyloxyphenyl-2′-hydroxy)-6-(3″-methylphenyl)-2-aminopyrimidine (**8o**)* Yield 29 %; Yellow crystals from MeOH; mp 165–167 °C; IR (KBr) νmax: 3450, 3330, 3200, 1607 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.1 (s, 3H, CH_3), 5.11 (s, 2H, OCH_2Ar), 6.2 (2H, NH_2), 7.2–7.4 (m, 5H, ArH), 6.9–7.8 (m, 7H, ArH), 7.35 (s, 1H, Pyri); MS *m/z*; 384.1 (M+1); Anal. Calcd for $C_{24}H_{21}N_3O_2$: C, 75.18; H, 5.52; N, 10.96. Found: C, 75.28; H, 5.51; N, 10.92.

Antiplasmodial activity

The chloroquine sensitive *P. falciparum* MRC 20 was obtained from the National Institute of Malaria Research (NIMR), New Delhi, India. The culture was synchronized

using sorbitol, and parasitemia was adjusted to 1–1.5 % by diluting with fresh human erythrocytes. The cells were diluted with complete media to make 8 % hematocrit. The slides of culture were prepared and observed for the calculation of parasitemia, particularly for young trophozoites or ring stages. One mg of each compound was dissolved in 100 μL dimethyl sulfoxide and 900 μL RPMI-1640 to obtain a stock of 1 mg/mL (stock solution). A series of eight concentrations were prepared from the stock solutions by twofold dilutions. After 24 h, thin films of the contents of each well were prepared and examined under the microscope. Parasite count for each blood film was made using a compound microscope under oil immersion with ×100 objective after staining the film with eosin yellow and methylene blue. Each film was observed at three different visual fields. The number of schizonts per 200 parasites were noted and compared between control and test wells for the determination of the percentage inhibition. All doses were studied in cultures and the mean was observed for the purpose of drawing inferences.

Supplementary data

Supplementary data associated with this article can be found in the online version.

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