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Solid support synthesis of 6-aryl-2-substituted pyrimidin-4-yl phenols as anti-infective agents

Anu Agarwal,[a] Kumkum Srivastava,[b] S. K. Puri,[b] S. Sinha[c] and Prem M. S. Chauhan[a,*]

[a]*Division of Medicinal & Process Chemistry, Central Drug Research Institute, Lucknow 226001, India*
[b]*Division of Parasitology, Central Drug Research Institute, Lucknow 226001, India*
[c]*Division of Biochemistry, Central Drug Research Institute, Lucknow 226001, India*

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Abstract—A library of 30 trisubstituted pyrimidines were synthesized and evaluated for their in vitro antimalarial and antitubercular activity. Out of the 30 compounds synthesized, 23 compounds have shown in vitro antimalarial activity against *Plasmodium falciparum* in the range of 0.25–2 μg/mL and 16 compounds have shown antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Ra$, at a concentration of 25 μg/mL.

Parasitic diseases have an overwhelming impact on public health in developing regions, and malaria has for a long time presented a serious global health problem. It is estimated that with 40% of the world's population exposed to the threat of malaria, there are an estimated 500 million clinical cases per year and 2 million deaths.[1] Out of the four species of plasmodium that affect humans, *Plasmodium falciparum* is the most prevalent and pathogenic. It causes Malaria tropica, which without treatment, is often lethal for the infected patient. Resistance of plasmodia to antimalarial drugs is now recognized as one of the major problems in eradication of malaria. Despite tremendous efforts an effective vaccine has not been found yet. The inadequate armory of drugs in widespread use for the treatment of malaria and lack of new drugs are the limiting factors in the fight against malaria. This underscores the continuing need for new drugs that attack crucial targets in the malarial pathogen. Pyrimethamine is a specific inhibitor of the plasmodial DHFR, which is one of the important targets for drugs against malaria. Inhibition of DHFR prevents biosynthesis of DNA, leading to cell death. The design of novel chemical entities specially affecting these targets could lead to better drugs for the treatment of malaria.[2,3]

In both lead identification and lead optimization processes, there is an acute need for new organic small molecules. Traditional methods of organic synthesis are orders of magnitude too slow to satisfy the demand for these compounds. To meet the increasing requirement of new compounds for drug discovery, speed is of essence, which can be met by combinatorial chemistry. Combinatorial chemistry is a widely accepted methodology that is used to generate libraries of molecules for discovery of biologically active leads and also the optimization of potential drug candidates. The mainstay of combinatorial chemistry is solid-phase organic synthesis. Nowadays combinatorial chemistry is mainly used to synthesize libraries of structurally diverse small organic molecules. Among small molecules, nitrogen heterocycles have received special attention as they belong to a class of compounds with proven utility in medicinal chemistry.[4]

As part of our ongoing program devoted to the synthesis of diverse heterocycles as anti-infective agents,[5] we had previously reported antimalarial activity in substituted triazines, pyrimidines, and quinolines.[6] Previously, we have reported solid supported synthesis of quinolones,[7] substituted pyrimidines,[8] and pyrimido[4,5-*d*]pyrimidines as anti-infective agents.[9] In this communication, we have synthesized trisubstituted pyrimidines on solid support as antimalarial and antimycobacterial agents.

Polymer-bound aldehyde (**1**) was synthesized by reacting Merrifield resin with 4-hydroxy benzaldehyde in DMF

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* Corresponding author. Tel.: +91 522 2262411; fax: +91 522 2623405; e-mail addresses: prem_chauhan_2000@yahoo.com; premsc58@hotmail.com

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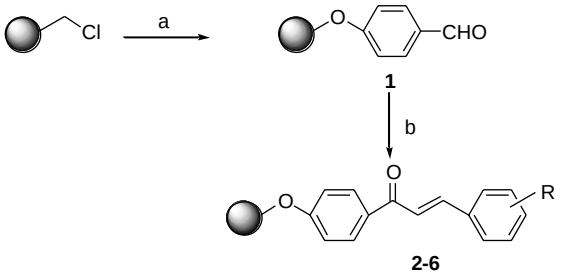
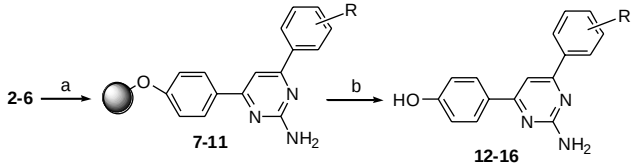
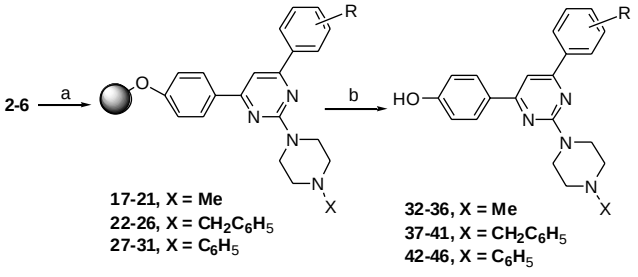
in the presence of sodium hydride at 80 °C for 40 h. The polymer-bound aldehyde was further reacted with different acetophenones to give polymer-bound chalcones (**2**–**6**) (Scheme 1).[10] The polymer-bound chalcones were reacted with different amidines in the presence of sodium methoxide in DMF at 80 °C for 30 h to give resin-bound pyrimidines (**7**–**11**, **17**–**26**, and **37**–**51**). The resin-bound pyrimidine derivatives were further subjected to cleavage in a 1:1 mixture of TFA and DCM to afford the final compounds (Schemes 2–4).[14]



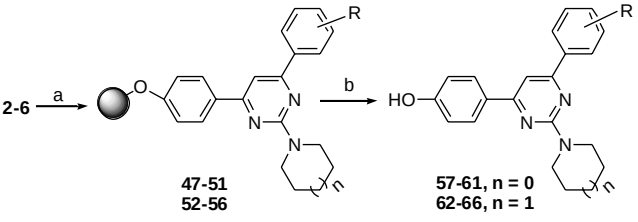
Scheme 1. Reagents and conditions: (a) 4-hydroxy benzaldehyde, NaH, DMF, 80 °C, 40 h; (b) different acetophenone, sodium methoxide, DMF, 48 h, rt.



Scheme 2. Reagents and conditions: (a) guanidine hydrochloride, sodium methoxide, DMF, 80 °C, 30 h; (b) DCM/TFA (1:1), 1 h.



Scheme 3. Reagents and conditions: (a) methyl or benzyl or phenyl piperazine-1-carboxamidine hydrochloride, sodium methoxide, DMF, 80 °C, 30 h; (b) DCM/TFA (1:1), 1 h.



Scheme 4. Reagents and conditions: (a) pyrrolidine or piperidine-1-carboxamidine hydrochloride, sodium methoxide, DMF, 80 °C, 30 h; (b) DCM/TFA (1:1), 1 h.

In Scheme 2 the chalcones **2**–**6** were cyclized with guanidine hydrochloride in the presence of sodium methoxide in DMF at 80 °C for 30 h to afford the polymer-bound compounds **7**–**11**. The mixture was then washed three times successively with DMF, water, methanol, DCM, and finally methanol to give resin-bound pyrimidines. The resin-bound compounds **7**–**11** were further subjected to cleavage in a 1:1 mixture of TFA and DCM to afford the final compounds **12**–**16**.

In Scheme 3 the chalcones **2**–**6** were cyclized with methyl, benzyl, and phenyl substituted piperazine-1-carboxamidine hydrochloride (synthesized by refluxing methyl, benzyl, or phenyl substituted piperazine with *S*-methylisothiourea sulfate in water[11]) in the presence of sodium methoxide in DMF by the same procedure as in Scheme 2 to afford the polymer-bound compounds **17**–**21**, **22**–**26**, and **27**–**31**, respectively, which further yielded compounds **32**–**46**.

In Scheme 4 the chalcones **2**–**6** were cyclized with piperidine or pyrrolidine-1-carboxamidine hydrochloride (synthesized by refluxing piperidine or pyrrolidine with *S*-methylisothiourea sulfate in water[11]) in the presence of sodium methoxide in DMF by the same procedure as in Scheme 2 to afford the polymer-bound compounds **47**–**51** and **52**–**56**. These pyrimidine derivatives **47**–**56** on cleavage gave compounds **57**–**66**. The synthesized compounds are well characterized by spectroscopic method as IR, mass, and NMR.[15]

The library was tested for its antimalarial activity[12] (against *P. falciparum* NF-54 strain) and antitubercular activity (against *M. tuberculosis* $H_{37}Ra$)[13] (Table 1).

In the library of trisubstituted pyrimidines, the variations have been done at the second and fourth position and the sixth position of pyrimidine ring is constant. Out of 30 synthesized compounds, 23 compounds have shown in vitro antimalarial activity in the range of 0.25–2 μg/mL. The compounds **12**–**16** having amino group at the second position of the pyrimidine ring have shown antimalarial activity at a concentration of 10 or 50 μg/mL. In compounds **32**–**36** having methyl piperazine at the second position of pyrimidine ring exhibited antimalarial activity in the range of 0.25–1 μg/mL. The compound **32** having phenyl ring at the fourth position of pyrimidine ring exhibited a MIC of 0.5 μg/mL. Substitution on the phenyl ring with methyl group (**33**) decreased the activity to 1 μg/mL whereas substitution with methoxy group (**34**) retained the activity having a MIC of 0.5 μg/mL. Disubstitution of methoxy group on the phenyl ring (**35**, **36**) further enhanced the activity having a MIC of 0.25 μg/mL. Replacing the methyl group of methyl piperazine with benzyl group (**37**) decreased the activity having a MIC of 1 μg/mL whereas replacement with phenyl group (**42**) further decreased the activity having a MIC of 2 μg/mL. This result shows the better efficacy of methyl piperazine over benzyl and phenyl piperazine. On substituting cyclic amines as pyrrolidine (**57**) and piperidine (**62**) the activity decreased having a MIC of 2 μg/mL. In general substituting the phenyl ring with methyl group decreased the activity

Table 1. Activity of trisubstituted pyrimidines

Compound	R	*P. falciparum* MIC (μg/mL)	*M. tuberculosis* MIC (μg/mL)
12	H	10	—
13	4-Me	50	—
14	4-OMe	50	—
15	3,4-DiOMe	10	12.5
16	2,5-DiOMe	10	25
32	H	0.5	25
33	4-Me	1	25
34	4-OMe	0.5	25
35	3,4-DiOMe	0.25	12.5
36	2,5-DiOMe	0.25	12.5
37	H	1	25
38	4-Me	2	—
39	4-OMe	1	—
40	3,4-DiOMe	0.5	—
41	2,5-DiOMe	0.25	25
42	H	10	25
43	4-Me	10	—
44	4-OMe	2	—
45	3,4-DiOMe	1	—
46	2,5-DiOMe	2	—
57	H	2	25
58	4-Me	2	—
59	4-OMe	1	—
60	3,4-DiOMe	1	25
61	2,5-DiOMe	1	25
62	H	2	25
63	4-Me	2	—
64	4-OMe	0.5	—
65	3,4-DiOMe	1	25
66	2,5-DiOMe	1	25

'—' inactive. MIC of pyrimethamine: 10 μg/mL.

whereas substitution with methoxy group increased the activity. Disubstituting the phenyl ring with methoxy group further increased the activity of compounds.

In antitubercular activity against *M tuberculosis* $H_{37}Ra$, 16 compounds out of the 30 compounds have shown activity at a concentration of 25 μg/mL. Three compounds have also shown >90% inhibition at a concentration of 12.5 μg/mL. In general, the compounds showed increase in activity on substituting the phenyl ring with methoxy group, whereas activity decreased on substitution with methyl group. Disubstitution on the phenyl ring with methoxy group further increased the activity. The compounds **12**–**16** having amino group at the second position of the pyrimidine ring, the compounds **12**, **13**, and **14** did not show any activity, whereas compounds **15** and **16** having dimethoxy substituted phenyl ring exhibited a MIC of 12.5 and 25 μg/mL, respectively. All compounds (**32**–**36**) having methyl piperazine at the second position of the pyrimidine ring have shown activity. The compound **32** having unsubstituted phenyl ring at the sixth position of pyrimidine ring has shown >90% inhibition at a concentration of 25 μg/mL. Substitution with methoxy (**34**) or methyl (**33**) group did not affect the activity, whereas disubstitution with methoxy groups (**35**, **36**) increased the activity having a MIC of 12.5 μg/mL. Replacement of the methyl group in methyl piperazine with benzyl (**37**–**41**) and phenyl group (**42**–**46**) decreased the activity. In compounds **57**–**61** and **62**–**66** having pyrrolidine and piperidine at the second position of the pyrimidine ring, only those compounds having unsubstituted phenyl ring or dimethoxy substituted phenyl ring at sixth position of the pyrimidine ring showed inhibition at a concentration of 25 μg/mL.

The thirty 2,4,6-trisubstituted pyrimidines were synthesized as pyrimethamine analogues. Out of the 30 synthesized compounds 23 compounds have shown antimalarial activity in the range of 0.25–2.0 μg/mL, whereas 16 compounds have shown antitubercular activity at a concentration of 25 μg/mL. These compounds are 5–40 times more potent than pyrimethamine. These identified pyrimidines are new leads in antimalarial chemotherapy. These molecules can be very useful for further optimization work in malarial chemotherapy.

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14. General experimental procedure: (a) Synthesis of polymer-bound aldehyde (**1**). A mixture of Merrifield resin (5 g, 1.6 mmol/g), 4-hydroxy benzaldehyde (5 g, 8 mol), NaH, and DMF (100 ml) was shaken at 80 °C for 40 h and then washed successively with DMF (30 ml, three times), MeOH (30 ml, three times), DCM (30 ml, three times), and finally MeOH (20 ml, two times). The polymer-bound aldehyde **1** was dried at 60 °C under high vacuum for 1 h. (b) Polymer-bound chalcones (**2**–**6**). The polymer-bound aldehyde (**1**, 1 g, 1.6 mmol/g) was further reacted with different acetophenones to give polymer-bound chalcones (**2**–**6**). The polymer-bound resin was first allowed to swell in DMF (20 ml) for 10 min and then substituted acetophenones (5 mmol) and sodium methoxide (5 mmol) were added. The reaction mixture was allowed to shake at room temperature for 48 h and then washed successively with water, DMF (10 ml two times), methanol (10 ml, two times), DCM (10 ml, two times), and finally with MeOH (10 ml, two times). Drying under high vacuum for 30 min afforded polymer-bound chalcones **2**–**6**. (c) Polymer-bound-4-(substituted-phenyl)-6-(4-alkyloxy-phenyl)-2-substituted-1-yl-pyrimidine (**7**–**11**, **17**–**31**, **47**–**56**). The polymer-bound chalcones (**2**–**6**, 50 mg, 1.6 mmol/g), substituted-1-carboxamidine hydrochloride (0.3 mmol), sodium methoxide (0.3 mmol) and DMF (10 ml) as solvent were heated and shaken at 80 °C for 30 h. The reaction was then washed successively with water, DMF, methanol, DCM, and finally with MeOH. Drying under high vacuum afforded polymer-bound pyrimidines. (d) 4-[6-(Substituted-phenyl)-2-substituted-1-yl-pyrimidin-4-yl]-phenol (**12**–**16**, **32**–**46**, **57**–**66**). The polymer-bound pyrimidines (**7**–**11**, **17**–**31**, **47**–**56**) were treated with a 1:1 mixture of TFA and DCM for 1 h and filtered. The filtrate was concentrated to afford a residue which on crystallized from methanol or from chloroform and hexane afforded the products **12**–**16**, **32**–**46**, **57**–**66**.
15. Spectroscopic data for **14**. MS: 300 (M+1); IR (KBr) 3387, 3242, 3030, 2933, 2841, 1641, 1601, 1511, 1451 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.07 (d, 2H, J = 8.8 Hz), 7.98 (d, 2H, J = 8.6 Hz), 7.30 (s, 1H), 7.00 (d, 2H, J = 8.8 Hz), 6.94 (d, 2H, J = 8.6 Hz), 3.86 (s, 3H, OMe). Spectroscopic data for **34**. MS : 377 (M+1); IR (KBr) 3429, 3032, 2934, 2855, 1635, 1603, 1508, 1460, 1356 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.08 (d, 2H, J = 8.8 Hz), 8.02 (d, 2H, J = 8.6 Hz), 7.28 (s, 1H), 6.99 (d, 2H, J = 8.8 Hz), 6.94 (d, 2H, J = 8.6 Hz), 4.05 (t, 4H, J = 4.6 Hz), 3.87 (s, 3H, OMe), 2.56 (t, 4H, J = 4.6 Hz), 2.38 (s, 3H, NMe). Spectroscopic data for **39**. MS: 453 (M+1); IR (KBr) 3375, 3034, 2955, 2851, 1592, 1543, 1447, 1362 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.07 (d, 2H, J = 8.6 Hz), 7.98 (d, 2H, J = 8.4 Hz), 7.38–7.33 (m, 5H), 7.29 (s, 1H), 6.99 (d, 2H, J = 8.6 Hz), 6.92 (d, 2H, J = 8.4 Hz), 4.03 (t, 4H, J = 4.4 Hz), 3.85 (s, 3H, OMe), 3.58 (s, 2H), 2.58 (t, 4H, J = 4.4 Hz). Spectroscopic data for **44**. MS: 439 (M+1); IR (KBr) 3265, 3028, 2952, 2856, 1604, 1579, 1509, 1441, 1344 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.09 (d, 2H, J = 8.6 Hz), 7.99 (d, 2H, J = 8.3 Hz), 7.30 (s, 1H), 6.95 (d, 2H, J = 8.3 Hz), 7.36 (d, 2H, J = 8.4 Hz), 7.03–6.97 (m, 5H), 4.19 (t, 4H, J = 4.8 Hz), 3.87 (s, 3H, OMe), 3.33 (t, 4H, J = 4.8 Hz). Spectroscopic data for **59**. MS: 348 (M+1); IR (KBr) 3226, 3027, 2933, 2839, 1605, 1577, 1541, 1448, 1356 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.11 (d, 2H, J = 8.8 Hz), 8.02 (d, 2H, J = 8.6 Hz), 7.36 (s, 1H), 7.00 (d, 2H, J = 8.8 Hz), 6.95 (d, 2H, J = 8.6 Hz), 3.86 (s, 3H, OMe), 3.74 (t, 4H, J = 5.8 Hz), 2.04 (t, 4H, J = 5.8 Hz). Spectroscopic data for **64**. MS: 362 (M+1); IR (KBr) 3243, 3032, 2926, 2840, 1595, 1505, 1439, 1356 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.08 (d, 2H, J = 8.7 Hz), 8.00 (d, 2H, J = 8.5 Hz), 7.30 (s,1H), 7.02 (d, 2H, J = 8.7 Hz), 6.96 (d, 2H, J = 8.5 Hz), 3.99 (t, 4H, J = 4.6 Hz), 3.87 (s, 3H, OMe), 1.68 (m, 6H).