Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 5218–5221
Bioorganic & Medicinal Chemistry Letters

A small library of trisubstituted pyrimidines as antimalarial and antitubercular agents

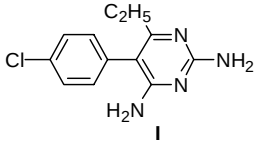
Anu Agarwal,[a] Kumkum Srivastava,[b] S. K. Puri,[b] S. Sinha[c] and Prem M. S. Chauhan[a,*]

[a]*Division of Medicinal and 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*

Received 4 July 2005; revised 12 August 2005; accepted 18 August 2005
Available online 19 September 2005

Abstract—A small library of 20 trisubstituted pyrimidines were synthesized and evaluated for their in vitro antimalarial and antitubercular activity. Out of the total screened compounds, 16 compounds have shown in vitro antimalarial activity against *Plasmodium falciparum* in the range of 0.25–2 μg/mL and 8 compounds have shown antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Ra$, at a concentration of 12.5 μg/mL.

Malaria is the most serious and widespread parasitic disease because of its prevalence, virulence and drug resistance, having an overwhelming impact on public health in developing regions of the world. Each year, more than 500 million people are infected and close to two million die because of malaria. *Plasmodium falciparum* is the main cause of severe clinical malaria and death. Endemic map indicates that *P. falciparum* and *Plasmodium vivax* account for 95% of the malaria infections.[1] There are a number of effective drugs available that interact in different ways with the biochemical life cycle of the parasite (quinine, chloroquine, primaquine, cycloguanil, pyrimethamine, and proguanil), but as the parasites rapidly develop permanent resistance against the different subclasses, there is a great urge to develop new and effective drugs attacking crucial targets in the metabolism of the malaria pathogen. Development of active and selective chemotherapeutic agents could be achieved by rational drug design taking into consideration the biochemical machinery of the parasite.[2] One of the targets for drugs against malaria is the enzyme dihydrofolate reductase (DHFR). DHFR has received considerable attention, as it is the target of pyrimethamine (**I**) and other antifolates used for the prophylaxis and treatment of *P. falciparum* infection. The role of DHFR is to catalyse the NADPH dependent reduction of dihydrofolate to give tetrahydrofolate, a central component in the single carbon metabolic pathway. The tetrahydrofolate is methylated to methylene tetrahydrofolate, which is directly involved in thymidine synthesis (assisting the methylation of deoxyuridine monophosphate to give thymidine monophosphate) and indirectly implicated in the metabolism of amino acids and purine nucleotide. Inhibition of DHFR thus prevents biosynthesis of DNA leading to cell death.[3]

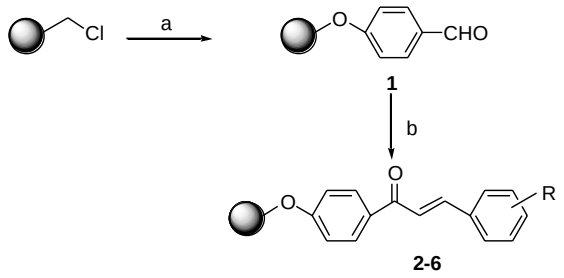


I

In both lead identification and lead optimization process 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 powerful tool to generate a large number of compounds for the screening of functional molecules. In the field of combinatorial chemistry, solid phase organic synthesis has been accepted as an efficient method for high throughput screening. In recent years the design and synthesis of structurally diverse

Keywords: Malaria; Dihydrofolate reductase; Solid phase synthesis; Combinatorial chemistry.
* Corresponding author. Tel.: +91 522 2262411; fax: +91 522 2623405; e-mail addresses: prem_chauhan_2000@yahoo.com; premsc58@hotmail.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.08.053



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

multifunctional libraries of small organic molecules on solid supports has been the central claim of combinatorial chemistry. Among small molecules heterocyclic structures have received special attention in combinatorial synthesis 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 in the presence of sodium hydride at 80 °C. The polymer bound aldehyde was further reacted with different substituted acetophenones to give polymer bound chalcones (**2**–**6**) (Scheme 1).[10] The polymer bound chalcones were reacted with different substituted amidines in the presence of sodium methoxide in DMF to give resin bound pyrimidines (7–11, 17–21, 22–26, and 37–41). 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). All the synthesized compounds are well characterized by spectroscopic method as IR, mass, NMR and elemental analysis.[14]

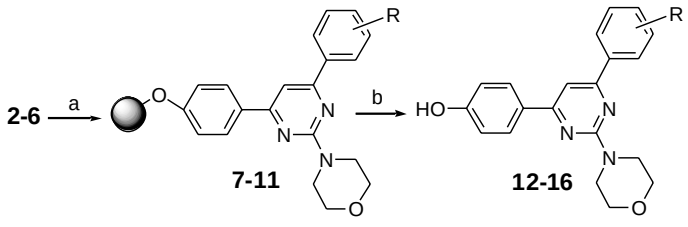
In Scheme 2 the chalcones **2**–**6** were cyclised with morpholine-4-carboxamidine hydrochloride (synthesized by refluxing morpholine with *S*-methylisothiourea sulphate in water[11]) in the presence of sodium methoxide in DMF at 80 °C for 30 h. The mixture was then three times washed successively with DMF, water, methanol, DCM and finally methanol to give resin bound pyrimidines **7**–**11**. The resin bound pyrimidine derivatives 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 cyclised with *N*-(3-morpholin-4-yl/3-imidazol-1-yl-propyl)-guanidine hydrochloride (synthesized by refluxing 3-morpholin-4-yl-propylamine/3-imidazol-1-yl-propylamine with *S*-methylisothiourea sulphate in water) in the presence of sodium methoxide in DMF by the same procedure as in Scheme 2 to afford the polymer bound compounds **17**–**26** which on cleavage gave compounds **27**–**36**.

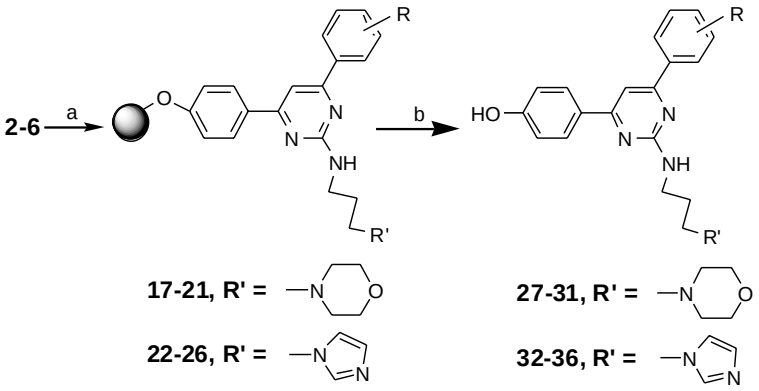
In Scheme 4 the chalcones **2**–**6** were cyclised with *N*-(4-diethylamino-1-methyl-butyl)-guanidine hydrochloride (synthesized by refluxing 2-amino-5-diethylamino pentane with *S*-methylisothiourea sulphate in water) in the presence of sodium methoxide in DMF by the same procedure as in Scheme 2 to afford the polymer bound compounds **37**–**41**which further yielded compounds **42**–**46**.

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

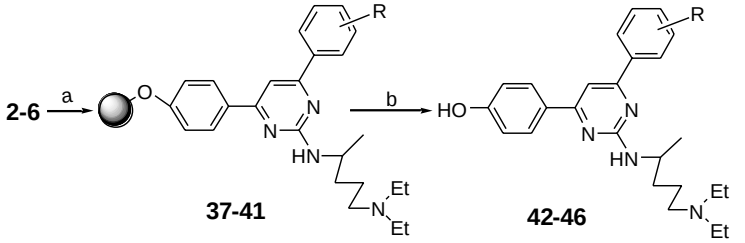
In the library of trisubstituted pyrimidines the variation have been done at the 2nd and 4th position and the 6th position of pyrimidine ring is constant. Out of 20 synthesized compounds, 16 compounds have shown in vitro antimalarial activity in the range of 0.25–2 μg/mL. In compounds (**12**–**16**) having morpholine ring at the 2nd position of pyrimidine ring, exhibited antimalarial activity in the range of 0.5–2 μg/mL. The compound **12** having phenyl ring at 4th position exhibited a MIC of 2 μg/mL. Substituting the phenyl ring with methyl group (**13**) had no effect on the activity whereas substitution with methoxy group (**14**) increased the activity having a MIC of 1 μg/mL. Disubstitution of methoxy group on the phenyl ring (**15**, **16**) further enhanced the activity having a MIC of 0.5 μg/mL. On further substitution at the 2nd position of pyrimidine ring with 4-(3-aminopropyl)morpholine, the compound **27** has shown a MIC of 2 μg/mL. Substituting the morpholine ring in compound **27** with imidazole ring the activity of compound **32** increased having a MIC of 0.5 μg/mL. In general on substituting the phenyl ring with methyl group



Scheme 2. Reagents and conditions: (a) morpholine-4-carboxamidine hydrochloride, sodium methoxide, DMF, 80 °C, 30 h. (b) DCM: TFA (1:1), 1 h.



Scheme 3. Reagents and conditions: (a) *N*-substituted-guanidine hydrochloride, sodium methoxide, DMF, 80 °C, 30 h. (b) DCM: TFA (1:1), 1 h.



Scheme 4. Reagents and conditions: (a) *N*-(4-diethylamino-L-methyl-butyl)-guanidine-hydrochloride, sodium methoxide, DMF, 0 °C, 30 h. (b) DCM: TFA (1:1), 1 h.

Table 1. Biological activity of trisubstituted pyrimidines

Compound	R	*P. falciparum* MIC (μg/mL)	*M. tuberculosis* MIC (μg/mL)
12	H	2	—
13	4-Me	2	—
14	4-OMe	1	—
15	3,4-diOMe	0.5	—
16	2,5-diOMe	0.5	—
27	H	2	12.5
28	4-Me	2	—
29	4-OMe	1	12.5
30	3,4-diOMe	0.5	25
31	2,5-diOMe	0.5	25
32	H	0.5	25
33	4-Me	2	—
34	4-OMe	1	12.5
35	3,4-diOMe	0.25	12.5
36	2,5-diOMe	0.25	12.5
42	H	10	25
43	4-Me	50	—
44	4-OMe	10	12.5
45	3,4-diOMe	2	12.5
46	2,5-diOMe	10	12.5

MIC of pyrimethamine, 10 μg/mL.

decreased the activity 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* $H_{37}Ra$, 12 compounds out of the 20 compounds have shown activity at a concentration of 25 μg/mL. Eight compounds have also shown activity at a concentration of 12.5 μg/mL. Compounds **12**–**16** having morpholine group at the 2nd position of the pyrimidine ring did not showed any activity. Replacing the morpholine group with 4-(3-aminopropyl)morpholine the compound **27** having phenyl group have shown a MIC of 12.5 μg/mL. Substituting the phenyl ring with methyl group (**28**) decreased the activity whereas substitution with methoxy group (**29**) retained the activity. Disubstitution with methoxy group (**30**, **31**) decreased the activity having a MIC of 25 μg/mL. In compounds (**32**–**36**) having 4-(3-aminopropyl)imidazole chain at the 2nd position of the pyrimidine ring the compound **32** have shown a MIC of 25 μg/mL. Disubstitution of the phenyl ring with dimethoxy group the compounds **35** and **36** exhibited a MIC of 12.5 μg/mL in contrast to compounds **30** and **31** where activity decreased on disubstitution of methoxy group. In compounds **42**–**46** the compounds **42** having phenyl ring showed a MIC of 25 μg/mL and activity increased on disubstituting the phenyl ring with methoxy group. In general the compounds showed increase in activity on substituting the phenyl ring at 6th position with methoxy group, whereas activity decreased on substitution with methyl group.

The twenty 2,4,6-trisubstituted pyrimidines were synthesized as pyrimethamine analogues. Out of the 20 synthesized compounds, 16 compounds have shown antimalarial activity in the range of 0.25–2.0 μg/mL, whereas 12 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 chemo-

therapy. These molecules can be very useful for further optimization work in malarial chemotherapy.

Acknowledgments

A.A. thanks the Council of Scientific and Industrial Research (India) for the award of Senior Research Fellowship. We are also thankful to S.A.I.F. Division, CDRI, Lucknow for providing spectroscopic data. CDRI Communication No. 6823.

References and notes

1. (a) Kumar, A.; Katiyar, S. B.; Agarwal, A.; Chauhan, P. M. S. *Curr. Med. Chem.* **2003**, *10*, 1137; (b) Shrivastava, S. K.; Chauhan, P. M. S. *Curr. Med. Chem.* **2001**, *8*, 1535.
2. Kumar, A.; Katiyar, S. B.; Agarwal, A.; Chauhan, P. M. S. *Drugs Future* **2003**, *28*(3), 243.
3. (a) Murray, M. C.; Perkins, M. E. *Annu. Rep. Med. Chem.* **1996**, *31*, 141; (b) Enserink, M. *Science* **2000**, *287*, 1956; (c) Vilaivan, V.; Saesaengseerung, N.; Jarprung, D.; Kamchonwongpaisan, S.; Sirawaraporne, W.; Yuthavong, Y. *Bioorg. Med. Chem.* **2003**, *11*, 217; (d) Rastelli, G.; Sirawaraporm, W.; Sompornpisut, P., et al. *Bioorg. Med. Chem.* **2000**, *8*, 1117.
4. Larhed, M.; Hallberg, A. *Drug Discov. Today* **2001**, *6*, 406.
5. (a) Srivastava, S. K.; Chauhan, P. M. S.; Agarwal, S. K.; Bhaduri, A. P.; Singh, S. N.; Fatima, N.; Chatterjee, R. K.; Bose, C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2623; (b) Srivastava, S. K.; Agarwal, A.; Chauhan, P. M. S.; Agarwal, S. K.; Bhaduri, A. P.; Singh, S. N.; Fatima, N.; Chatterjee, R. K. *J. Med. Chem.* **1999**, *42*, 1667; (c) Srivastava, S. K.; Agarwal, A.; Chauhan, P. M. S.; Agarwal, S. K.; Bhaduri, A. P.; Singh, S. N.; Fatima, N.; Chatterjee, R. K. *Bioorg. Med. Chem.* **1999**, *7*, 1223; (d) Srivastava, S. K.; Chauhan, P. M. S.; Bhaduri, A. P.; Fatima, N.; Chatterjee, R. K. *J. Med. Chem.* **2000**, *43*, 2275; (e) Tiwari, S.; Chauhan, P. M. S.; Bhaduri, A. P.; Fatima, N.; Chatterjee, R. K. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1409.
6. (a) Agarwal, A.; Srivastava, K.; Puri, S. K.; Chauhan, P. M. S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 531; (b) Shrivastava, S.; Tiwari, S.; Chauhan, P. M. S.; Puri, S. K.; Bhaduri, A. P.; Pandey, V. C. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 653; (c) Shrivastava, S.; Tiwari, S.; Shrivastava, S. K.; Chauhan, P. M. S.; Bhaduri, A. P.; Pandey, V. C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2741.
7. Srivastava, S. K.; Haq, W.; Murthy, P. K.; Chauhan, P. M. S.; Murthy, P. K. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1885.
8. (a) Chauhan, P. M. S.; Kumar, A. *Comb. Chem. High Throughput Screen.* **2002**, *5*, 93; (b) Kumar, A.; Sinha, S.; Chauhan, P. M. S. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 667; (c) Srivastava, S. K.; Haq, W.; Chauhan, P. M. S. *Comb. Chem. High Throughput Screen.* **1999**, *2*, 33.
9. Srivastava, S. K.; Haq, W.; Chauhan, P. M. S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 965.
10. Katritzky, A. R.; Chassaing, C.; Barrow, S. J.; Zhang, Z.; Vvedensky, V.; Forood, B. *J. Comb. Chem.* **2002**, *4*, 249.
11. Andrews, K. J. M.; Anand, N.; Todd, A. R.; Topham, A. *J. Chem. Soc.* **1949**, 2490.
12. (a) Rieckmann, K. H.; Sax, L. J.; Campbell, G. H. *Mrema Lancet* **1978**, *1*, 22; (b) Trager, W.; Jensen, J. B. *Science* **1979**, *193*, 673; (c) Lambros, C.; Vanderberg, J. P. *J. Parasitol.* **1979**, *65*, 418.
13. Collins, L. A.; Franzblan, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004.
14. Spectroscopic data for **14**: Yield: 76%; HPLC Purity: 98.5; MS : 364 (M + 1); IR (KBr) 3236, 3028, 2967, 2855, 1628, 1511, 1441, 1358 cm^{-1};^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.09 (d, 2H, J = 8.8 Hz), 7.99 (d, 2H, J = 8.6 Hz), 7.33 (s, 1H), 7.01 (d, 2H, J = 8.8 Hz), 6.95 (d, 2H, J = 8.6 Hz), 3.98 (t, 4H, J = 4.5 Hz), 3.88 (s, 3H, OMe), 3.82 (t, 4H, J = 4.5H). Spectroscopic data for **29**: Yield: 74%; HPLC Purity: 96.2; MS : 421 (M + 1); IR (KBr) 3429, 3031, 2929, 2864, 1589, 1510, 1460, 1359 cm^{-1};^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.09 (d, 2H, J = 8.8 Hz), 8.01 (d, 2H, J = 8.5 Hz), 7.34 (s, 1H), 7.02 (d, 2H, J = 8.8Hz), 6.95 (d, 2H, J = 8.5Hz), 3.89 (s, 3H, OMe), 3.78 (t, 4H, J = 4.5Hz), 3.74 (t, 2H, J = 5.4H), 2.63 (t, 2H, J = 4.5 Hz), 2.56 (t, 4H, J = 4.6 Hz), 1.96–1.91 (m, 2H). Spectroscopic data for **34**: Yield: 70%; HPLC Purity: 98.9; MS: 402 (M + 1); IR (KBr) 3439, 3032, 2933, 2856, 1598, 1509, 1462, 1350 cm^{-1};^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.06 (d, 2H, J = 8.7 Hz), 8.00 (d, 2H, J = 8.5 Hz), 7.53 (s, 1H), 7.30 (s, 1H), 7.07 (d, 1H, J = 4.5 Hz), 7.00 (d, 2H, J = 8.7 Hz), 6.98 (d, 1H, J = 4.5 Hz), 6.92 (d, 2H, J = 8.5 Hz), 4.12 (t, 4H, J = 4.7 Hz), 3.87 (s, 3H, OMe), 3.60 (t, 4H, J = 4.7Hz), 2.22 (m, 2H). Spectroscopic data for **44**: Yield: 65%; HPLC Purity: 89.5; MS: 435 (M + 1); IR (KBr) 3427, 3031, 2930, 2864, 1594, 1442, 1356 cm^{-1};^{1}H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.08 (d, 2H, J = 8.8 Hz), 8.01 (d, 2H, J = 8.6 Hz), 7.28 (s, 1H), 6.99 (d, 2H, J = 8.8 Hz), 6.92 (d, 2H, J = 8.6 Hz), 3.88 (s, 3H, OMe), 3.57 (m, 1H), 3.24 (m, 2H), 2.88 (m, 2H), 2.53 (m, 4H), 1.32 (m, 2H), 1.05 (t, 6H).