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Bioorganic & Medicinal Chemistry Letters 15 (2005) 1881–1883
Bioorganic & Medicinal Chemistry Letters

Antimalarial activity of 2,4,6-trisubstituted pyrimidines

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Received 22 December 2004; revised 1 February 2005; accepted 4 February 2005

Abstract—A series of 2,4,6-trisubstituted pyrimidines (**3a**–**o**) was synthesized and evaluated for their in vitro antimalarial activity against *P. falciparum*. Out of the 15 compounds synthesized 11 compounds showed MIC in the range of 0.5–2 μg/mL. These compounds are in vitro several folds more active than pyrimethamine.

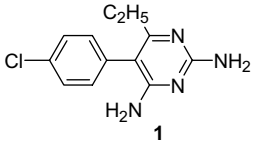
1. Introduction

Malaria is one of the most widespread diseases in the world. Each year, more than 100 million people are infected and close to two million die, because they do not receive adequate treatment.[1–3] Malaria is caused by different species of Plasmodium, of which *P. falciparum* is the most vicious one. It causes Malaria tropica, which without treatment, is often lethal for the infected patient. 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.[4] Pyrimethamine is a specific inhibitor of the plasmodial DHFR, which is one of the important target for drugs against malaria. The role of DHFR is to catalyze 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 implicated in the metabolism of amino acids and purine nucleotide. Inhibition of DHFR thus 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.[5,6]

As part of our ongoing program devoted to the synthesis of diverse heterocycles as anti-infective agents,[7] we had previously reported antimalarial activity in substituted triazines and quinolines.[8] In this study we report a new prototype, having pyridine moiety along with pyrimidine moiety as a new class for antimalarial activity.

2. Chemistry

To synthesize the 2,4,6-trisubstituted pyrimidine compounds (**3**), 4-acetylpyridine was reacted with different aldehydes (**a**–**o**) in 10% aq NaOH and methanol to yield the corresponding chalcones **2**(**a**–**o**).[9] Morpholine-4-carboxamidine hydrochloride was synthesized by refluxing morpholine with *S*-methylisothiourea sulfate in water according to a reported procedure.[10a] The chalcones **2a**–**o** were further cyclized with morpholine-4-carboxamidine hydrochloride in the presence of sodium isopropoxide (synthesized in situ by adding sodium metal in isopropanol) to afford the 2,4,6-trisubstituted pyrimidines **3**(**a**–**o**) as shown in Scheme 1. In the cyclization



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Keywords: Dihydrofolate reductase; Antimalarial; Pyrimidine.
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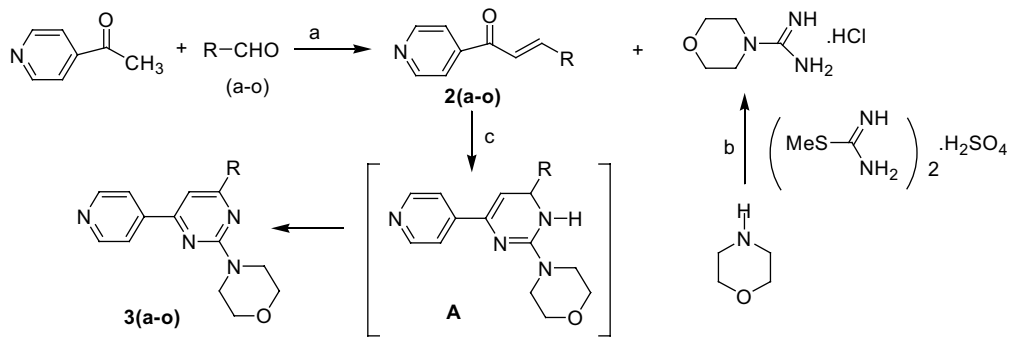
doi:10.1016/j.bmcl.2005.02.015



Scheme 1. Reagents and conditions: (a) different aldehydes, 10% aq NaOH, methanol, 0 °C to rt, 30 min; (b) (i) morpholine, *S*-methylisothiourea sulfate, water, reflux, 15 min; (ii) barium chloride, reflux, 15 min; (c) morpholine-4-carboxamidine. HCl, sodium isopropoxide, isopropanol, reflux, 8 h.

initially dihydropyrimidine (**A**) is formed, which is oxidized in the presence of air to form the pyrimidine.[10b] All the synthesized compounds were well characterized by spectroscopic methods as IR, mass, NMR and elemental analysis.[14]

3. Biological activity

The in vitro antimalarial assay was carried out in 96 well microtiter plates according to the micro assay of Rieckmann.[11] The culture of *P. falciparum* NF-54 strain is routinely being maintained in medium RPMI-1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum.[12] The asynchronous parasite of *P. falciparum* was synchronized after 5% D-Sorbitol treatment to obtain parasitized cells harbouring only the ring stage.[13] For carrying out the assay, an initial ring stage parasitaemia of ≈1% at 3% hematocrit in total volume of 200 μL of medium RPMI-1640 was uniformly maintained. The test compound in 20 μL volume at the required concentration (ranging between 0.25 μg and 50 μg/mL) in duplicate wells, were incubated with parasitized cell preparation at 37 °C in candle jar. After 36–40 h incubation, the blood smears from each well were prepared and stained with giemsa stain. The slides were microscopically observed to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of compounds. The tested concentration, which inhibits the complete maturation into schizonts, was recorded as the minimum inhibitory concentration (MIC). Pyrimethamine was used as the standard reference drug. Activity of all the tested compounds is shown in Table 1.

4. Results and discussion

Among all the 15 compounds tested, 1 compound showed MIC of 0.5 μg/mL whereas 6 compounds showed MIC of 1 μg/mL and 4 compounds have shown MIC, 2 μg/mL. The compounds showed a good structure–activity relationship. Most of the compounds showed a good correlation with Clog *P* value. In general compounds showed increase in activity with the decrease in Clog *P* value (lipophilicity).

Table 1. Antimalarial in vitro activity against *P.falciparum*

Compd no.	R	Clog *P*[a]	MIC
3a	C_6H_5	3.07	2
3b	4-OMe-C_6H_4	3.11	2
3c	3-OMe-C_6H_4	3.11	2
3d	2,3-DiOMe-C_6H_3	2.56	1
3e	2,5-DiOMe-C_6H_3	2.61	1
3f	3,5-DiOMe-C_6H_3	3.17	2
3g	2,4,5-TriOMe-C_6H_2	2.31	0.5
3h	3,4,5-TriOMe-C_6H_2	2.52	10
3i	4-Me-C_6H_4	3.57	10
3j	4-SMe-C_6H_4	3.67	10
3k	3,4-DiMe-C_6H_3	4.02	1
3l	4OMe-naphthyl	4.28	1
3m	4Cl-C_6H_4	3.78	10
3n	3NO_2-C_6H_4	2.82	1
3o	2-Thiophene	2.75	1
1			10

MIC = Minimum inhibiting concentration for the development of ring stage parasite into the schizont stage during 40 h incubation.
1: standard drug, pyrimethamine.
[a] Clog *P* value were calculated using the software chemdraw.

Phenyl substituted compound (**3a**) having a Clog *P* value of 3.07, showed a MIC of 2 μg/mL. Substituting the phenyl ring with methoxy group at the 4 position (**3b**) and 3 position (**3c**) showed a Clog *P* value of 3.11 and a MIC of 2 μg/mL. In dimethoxy substituted compounds, 3,5-dimethoxy substituted compound (**3f**) has a Clog *P* value of 3.17 and a MIC of 2 μg/mL. 2,3-Dimethoxy substituted (**3d**) and 2,5-dimethoxy substituted (**3e**) compounds have a Clog *P* value of 2.56 and 2.61, respectively, and a MIC of 1 μg/mL. The 2,4,5-trimethoxy substituted compound (**3g**) have Clog *P* of 2.31 and a MIC of 0.5 μg/mL. All these results emphasize that with decrease in Clog *P* value activity increases. An exception to this, the 3,4,5-trimethoxy substituted compound (**3h**), showed a Clog *P* value of 2.52 and a MIC of 10 μg/mL. On substituting the phenyl ring with methyl (**3i**) and *S*-methyl group (**3j**) the Clog *P* value increased to 3.57 and 3.67, respectively, thus showing a MIC value of 10 μg/mL. Exceptions to this were compounds having R as 3,4-dimethylbenzene (**3k**) and 4-methoxynaphthalene (**3l**), having a Clog *P* value of 4.02 and 4.28, respectively, showing a MIC of 1 μg/mL. Substitution of chloro group at 4 position (**3m**) increased the Clog *P* value to 3.78 and thus the compound showed a MIC of 10 μg/mL.

Substitution of nitro group at 3 position (**3n**) reduced the Clog *P* value to 2.82, thus showing a MIC of 1 μg/mL. Substituting the benzene ring with thiophene ring (**3o**) also reduced the Clog *P* value to 2.75 having a MIC of 1 μg/mL. In general the compounds having Clog *P* value in the range of 2.56–2.82 showed a MIC of 1 μg/mL, whereas compounds having Clog *P* in the range 3.07–3.17 showed a MIC of 2 μg/mL. Compounds having Clog *P* value in the range of 3.57–3.78 showed a MIC value of 10 μg/mL. The interaction of the drug with the target has a complex nature so besides lipophilicity various other factors also contribute to the activity of the compound.

5. Conclusion

The fifteen 2,4,6-trisubstituted-pyrimidines (**3a**–**o**) were synthesized as pyrimethamine analogs. Out of the synthesized compounds one compound have shown MIC of 0.5 μg/mL. Six compounds showed MIC of 1 μg/mL, whereas four compounds showed MIC of 2 μg/mL. These compounds are 5–20 times more potent than pyrimethamine. The present study suggested that the newly synthesized 2,4,6-trisubstitued pyrimidines are new leads in antimalarial chemotherapy. These molecules can be very useful for further optimization work in malarial chemotherapy.

Acknowledgements

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. C.D.R.I. Communication No. 6702.

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14. Spectroscopic data for **3e** MS: 379 (M+1), mp 142–144 °C; IR (KBr) 2932, 1645, 1574, 1484, 1319, 1280 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.77 (d, 2H, J = 5.9 Hz), 7.95 (d, 2H, J = 5.9 Hz), 7.38 (s, 1H), 7.32 (s, 1H), 7.22 (d, 1H, J = 6.8 Hz), 6.71 (d, 1H, J = 6.8 Hz), 4.01 (t, 4H, J = 4.6 Hz), 3.87 (t, 4H, J = 4.6 Hz), 3.84 (s, 3H, OMe), 3.82 (s, 3H, OMe). ^{13}C ($CDCl_3$, 50 MHz): 164.9, 162.6, 162.2, 154.3, 152.9, 150.8, 146.1, 128.3, 121.6, 116.9, 116.6, 113.7, 107.9, 67.3, 56.9, 56.3, 44.8. Anal. Calcd for $C_{21}H_{22}N_4O_3$: C, 66.65 H, 5.86; N, 14.81. Found: C, 66.78; H, 5.98; N, 14.72. Spectroscopic data for **3g** MS: 409 (M+1), mp 153–155 °C; IR 2926, 1638, 1580, 1480, 1325, 1272 (KBr) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.75 (d, 2H, J = 6.1 Hz), 7.95 (d, 2H, J = 6.1 Hz), 7.80 (s, 1H), 7.72 (s, 1H), 6.62 (s, 1H), 3.97 (s, 3H, OMe), 4.02 (t, 4H, J = 4.6 Hz), 3.93 (s, 6H, 2OMe), 3.81 (t, 4H, J = 4.6 Hz). ^{13}C ($CDCl_3$, 50 MHz): 164.5, 162.6, 162.1, 154.1, 152.3, 150.8, 146.3, 143.9, 121.6, 118.9, 114.2, 107.6, 98.3, 67.3, 57.2, 57.0, 56.6, 44.8. Anal. Calcd for $C_{22}H_{24}N_4O_4$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.74; H, 5.86; N, 13.62. Spectroscopic data for **3k** MS: 347 (M+1), mp 194–196 °C; IR 2948, 1636, 1584, 1486, 1325, 1265 (KBr) cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.77 (d, 2H, J = 6.1 Hz), 7.97 (d, 2H, J = 6.1 Hz), 7.87 (s, 1H), 7.82 (d, 1H, J = 6.9 Hz), 7.42 (s, 1H), 7.23 (d, 1H, J = 6.9 Hz), 4.04 (t, 4H, J = 4.7 Hz), 3.84 (t, 4H, J = 4.7 Hz), 2.37 (s, 3H), 2.34 (s, 3H). ^{13}C ($CDCl_3$, 50 MHz): 166.6, 162.9, 162.7, 150.8, 145.9, 140.2, 137.4, 135.6, 130.5, 128.6, 125.0, 121.5, 102.6, 67.4, 44.8, 20.3, 20.1. Anal. Calcd for $C_{21}H_{22}N_4O$: C, 72.81; H, 6.40; N, 16.17. Found: C, 72.68; H, 6.22; N, 16.44. Spectroscopic data for **3l** MS: 399 (M+1), mp 160–162 °C; IR (KBr) 2936, 1642, 1578, 1488, 1324, 1284 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.76 (d, 2H, J = 6.1 Hz), 8.37–8.32 (m, 2H), 7.96 (d, 2H, J = 6.1 Hz), 7.70 (d, 1H, J = 8.0 Hz), 7.55–7.52 (m, 2H), 7.30 (s, 1H), 6.92 (d, 1H, J = 8.0 Hz), 4.07 (s, 3H, OMe), 4.02 (t, 4H, J = 4.6 Hz), 3.82 (t, 4H, J = 4.6 Hz). ^{13}C ($CDCl_3$, 50 MHz): 165.3, 162.5, 162.2, 161.9, 150.9, 145.5, 133.2, 129.9, 128.7, 127.6, 127.1, 125.9, 125.5, 122.9, 121.5, 107.6, 103.7, 67.4, 56.1, 44.9. Anal. Calcd for $C_{24}H_{22}N_4O_2$: C, 72.34; H, 5.57; N, 14.06. Found: C, 72.45; H, 5.65; N, 14.25.