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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 15 (2005) 3130–3132

Antimalarial activity and synthesis of new trisubstituted pyrimidines

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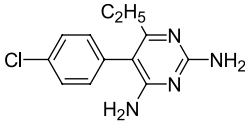
Received 24 February 2005; accepted 8 April 2005
Available online 4 May 2005

Abstract—A series of 2,4,6-trisubstituted-pyrimidines was synthesized and evaluated for their in vitro antimalarial activity against *Plasmodium falciparum*. Out of the 30 compounds synthesized 21 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 major problems of many tropical and subtropical countries in the world. 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. Malaria is caused by protozoan parasites, namely *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae*, and is transmitted to humans by female mosquitoes belonging to the genus *Anopheles*. Endemic maps indicates that *P. falciparum* and *P. vivax* account for 95% of malaria infections.[1,2] 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.[3] One of the targets for drugs against malaria is the enzyme dihydrofolate reductase (DHFR). Pyrimethamine (**I**) is a specific inhibitor of the plasmodial DHFR, which is essential for the DNA synthesis. The design of novel chemical entities specially affecting these targets could lead to better drugs for the treatment of malaria.[4–6]



I

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, pyrimidines, and quinolines.[8]

2. Chemistry

To synthesize the 2,4,6-trisubstituted-pyrimidine compounds (**3**), 4-acetylpyridine (**1**) was reacted with different aldehydes (**a–j**) in 10% aq-NaOH and methanol to yield the corresponding chalcones **2**(**a–j**).[9] Methyl, benzyl, and phenyl substituted piperazine-1-carboxamidine hydrochloride (**7x**) were synthesized by refluxing either methyl, benzyl, and phenyl piperazine (**6x**) with *S*-methyl-isothiourea sulfate in water according to a reported procedure.[10] The chalcones (**2a–j**) were further cyclized with different imidine hydrochlorides (**7x**) 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–j**), **4**(**a–j**), and **5**(**a–j**) as shown in Scheme 1. All the synthesized compounds were well characterized by spectroscopic data as IR, mass, NMR, and elemental analysis.[14]

3. Biological activity

The in vitro antimalarial assay was carried out in 96 well micro-titre plates according to the micro-assay of

Keywords: Dihydrofolate reductase; Antimalarial; Pyrimidine.
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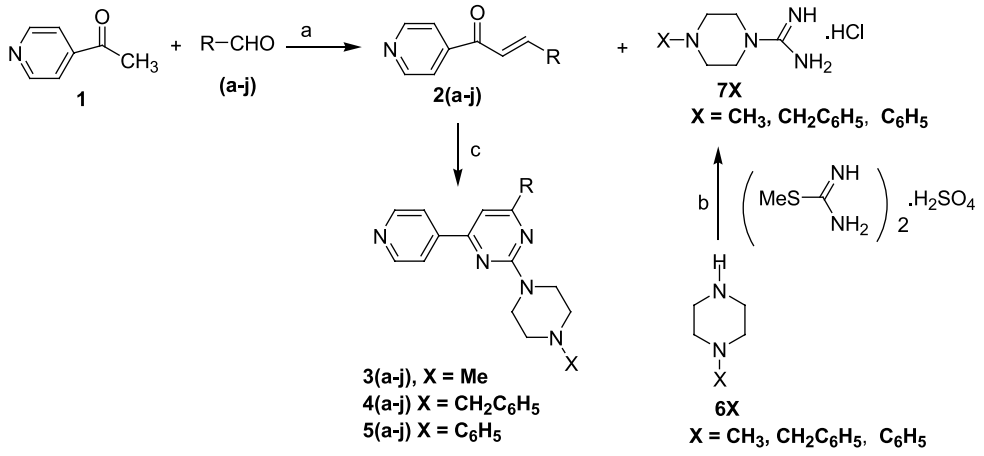
doi:10.1016/j.bmcl.2005.04.014



Scheme 1. Reagents and conditions: (a) Different aldehydes, 10% aq-NaOH, methanol, 0 °C–rt, 30 min. (b) (i) Different substituted piperazines, *S*-methyl-isothiourea sulfate, water, reflux, 15 min; (ii) Barium chloride, reflux, 15 min. (c) Substituted piperazine carboxamidine, HCl, sodiumisopropoxide, isopropanol, reflux, 8 h.

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 harboring only the ring stage.[13]

For carrying out the assay, an initial ring stage parasitaemia of ≈1% at 3% haematocrit in total volume of 200 μL of medium RPMI-1640 was uniformly maintained. The test compound in 20 μL volume at 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 test 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.

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

S. no.	R	MIC (μg/mL)		
		3(a–j) X = CH_3	**4(a–j)** X = CH_2Ph	**5(a–j)** X = C_6H_5
a	C_6H_5	1	2	10
b	4-Me-C_6H_4	1	2	1
c	4-SMe-C_6H_4	1	2	10
d	3,4-DiMe-C_6H_3	0.5	1	2
e	4OMe-C_6H_4	1	1	2
f	2,5-diOMe-C_6H_3	0.5	1	2
g	2,4,5-triOMe-C_6H_2	2	2	10
h	3,4,5-triOMe-C_6H_2	10	10	50
i	3NO_2-C_6H_4	1	2	10
j	4Cl-C_6H_4	2	10	10
I	Pyrimethamine		10	

MIC = Minimum inhibiting concentration for the development of ring stage parasite into the schizont stage during 40 h incubation.
I: standard drug, pyrimethamine.

4. Results and discussion

Among all the 30 compounds tested, 2 compounds showed MIC of 0.5 μg/mL whereas 9 compounds showed MIC of 1 μg/mL and 10 compounds have shown MIC, 2 μg/mL. The compounds showed a good structure activity relationship. When R was phenyl group then compounds **3a**, **4a**, and **5a** showed inhibition at a concentration of 1 μg/mL, 2 μg/mL, and 10 μg/mL, respectively. The activity was highest when the X group was methyl. On substituting the methyl group (**3a**) with benzyl group (**4a**), the MIC reduced slightly to 2 μg/mL where as on substitution with phenyl group (**5a**) there was a sharp decrease in the activity and the MIC dropped to 10 μg/mL. Substitution on the phenyl ring with methyl group (**3b**), thiomethyl group (**3c**), and methoxy group (**3e**) at 4-position the activity was retained at 1 g/mL. When the phenyl ring was di substituted with methyl group at 3 and 4 position (**3d**) or at 2 and 5 positions with methoxy group (**3f**) the activity increased having a MIC of 0.5 μg/mL. This shows that disubstitution on the phenyl ring favors the activity. Where as when the phenyl ring was triply substituted with methoxy group at 2,4,5 positions (**3g**) or 3,4,5 positions (**3h**) the activity reduced to 2 μg/mL and 10 μg/mL, respectively. This emphasizes the fact that trisubstitution do not favor the activity. Substituting the phenyl ring with nitro group at 3 position (**3i**) and chloro group at 4 position (**3j**) showed a MIC of 1 μg/mL and 2 μg/mL, respectively. In most of the cases (few exceptions as **5b**) compounds having X as methyl, benzyl, and phenyl group the activity reduced when the methyl group was substituted with benzyl group and further decreased when it was replaced with phenyl group.

5. Conclusion

The thirty 2,4,6-trisubstituted-pyrimidines **3(a–j)**, **4(a–j)**, and **5(a–j)** were synthesized as pyrimethamine analogues. Out of the synthesized compounds, two compounds have shown MIC of 0.5 μg/mL. Nine compounds showed MIC of 1 μg/mL, whereas 10 compounds showed MIC of 2 μg/mL. These compounds are 5–20 times more potent than pyrimethamine. These identified pyrimidines are new lead 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. CDRI Communication No. 6712.

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14. Spectroscopic data for **3e**: MS: 362(M+1); M.P. 152–154 °C; IR (KBr) 2932, 1645, 1574, 1484, 1319, 1280 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.75 (d, 2H, J = 5.9 Hz), 8.09 (d, 2H, J = 8.4 Hz), 7.95 (d, 2H, J = 5.9 Hz), 7.35 (s, 1H), 7.01 (d, 2H, J = 8.4 Hz), 4.05 (t, 4H, J = 4.8 Hz), 3.88 (s, 3H, OMe), 2.54 (t, 4H, J = 4.8 Hz), 2.37 (s, 3H, NMe). ^{13}C ($CDCl_3$, 50 MHz): 165.8, 162.8, 162.6, 162.2, 150.8, 146.1, 130.6, 129.0, 121.5, 114.5, 101.6, 55.8, 55.5, 46.7, 44.2. Anal. Calc for $C_{21}H_{23}N_5O$: Calculated C: 69.78, H: 6.41, N: 19.38. Found: C: 69.92, H: 6.54, N: 19.51. Spectroscopic data for **4e**: MS: 438(M+1); M.P. 148–150 °C; IR (KBr) 2926, 1638, 1580, 1480, 1325, 1272 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.74 (d, 2H, J = 6.0 Hz), 8.09 (d, 2H, J = 8.8 Hz), 7.94 (d, 2H, J = 6.0 Hz), 7.36 (s, 1H), 7.33–7.25 (m, 5H), 6.99 (d, 2H, J = 8.8 Hz), 4.03 (t, 4H, J = 4.8 Hz), 3.87 (s, 3H, OMe), 3.58 (s, 2H), 2.57 (t, 4H, J = 4.8 Hz). ^{13}C ($CDCl_3$, 50 MHz): 165.8, 162.8, 162.6, 162.2, 150.8, 146.2, 138.4, 130.6, 129.6, 129.0, 128.7, 127.6, 121.5, 114.5, 101.5, 63.6, 55.8, 53.5, 44.3. Anal. Calc for $C_{27}H_{27}N_5O$: Calculated C: 74.12, H: 6.22, N: 16.01. Found: C: 74.25, H: 6.34, N: 16.15. Spectroscopic data for **5e** MS: 424(M+1); M.P. 158–160 °C; IR (KBr) 2948, 1636, 1584, 1486, 1325, 1265 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.76 (d, 2H, J = 6.0 Hz), 8.11 (d, 2H, J = 8.7 Hz), 7.95 (d, 2H, J = 6.0 Hz), 7.37 (s, 1H), 7.26 (d, 2H, J = 8.4 Hz), 7.01 (d, 2H, J = 8.7 Hz), 6.93–6.86 (m, 3H), 4.18 (t, 4H, J = 4.7 Hz), 3.88 (s, 3H, OMe), 3.31 (t, 4H, J = 4.7 Hz). ^{13}C ($CDCl_3$, 50 MHz): 165.9, 162.9, 162.6, 162.3, 151.9, 150.8, 146.1, 130.5, 129.6, 129.1, 121.5, 120.6, 116.9, 114.5, 101.9, 55.8, 49.9, 44.3. Anal. Calc for $C_{26}H_{25}N_5O$: Calculated C: 73.74, H: 5.95, N: 16.54. Found: C: 73.88, H: 5.87, N: 16.71.