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Synthesis of 2-[3,5-substituted pyrazol-1-yl]-4,6-trisubstituted triazine derivatives as antimalarial agents

Sanjay Babu Katiyar,[a] Kumkum Srivastava,[b] S. K. Puri[b] 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*

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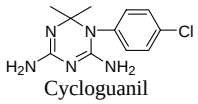
Abstract—A series of 22 compounds were synthesized and screened against *Plasmodium falciparum* NF-54 strain. Of the screened compounds, 6 compounds showed MIC in the range between 1 and 2 μg/mL. These compounds are 32 times more potent than the cycloguanil which was used as the standard drug.

About one-third of the world's population are currently living with a serious risk of contracting malaria.[1] It is estimated that there are approximately 2–3 million deaths every year from malaria,[2] and in sub-Saharan part of Africa alone about 1–2 million children below the age of 5 years die of malaria every year.[3] Despite continuous research efforts of more than two decades, no vaccine is yet discovered for effective control of malaria. Treatment of malaria is becoming more difficult due to the spreading resistance of the parasite to standard antimalarial drugs, in particular to chloroquine (CQ), which had been the affordable and effective antimalarial mainstay for more than 50 years,[4,5] to the resistance of mosquitoes to insecticides, and due to climatic changes that have enlarged areas of disease transmission. It is commonly known that infection caused by *Plasmodium falciparum* is frequently more fatal in children than in adults, producing respiratory distress, neurological problems, and severe anemia, and leading to death in 5–35% of severe infections. 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.[6] Cycloguanil and pyrimethamine are specific inhibitors of the plasmodial DHFR, which is one of the important targets 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 in 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.[7,8]

Previously, we had reported the antimalarial activity in the substituted triazines, pyrimidines, indoles, and quinolines.[9] Here, we report the antimalarial activity of new substituted pyrazolyl triazine derivatives.



Cycloguanil

The synthesis of targeted compounds **4**–**25** is shown in Scheme 1. The compound 3,3-bis-methylsulfanyl-1-(substituted-phenyl)-propenone (**1**) was synthesized by the reaction of substituted acetophenone with CS_2 in the presence of NaH followed by methylation with

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* Corresponding author: Tel.: +91 522 2262411x4332; fax: +91 522 2623405; e-mail: Prem_chauhan_2000@yahoo.mail.com

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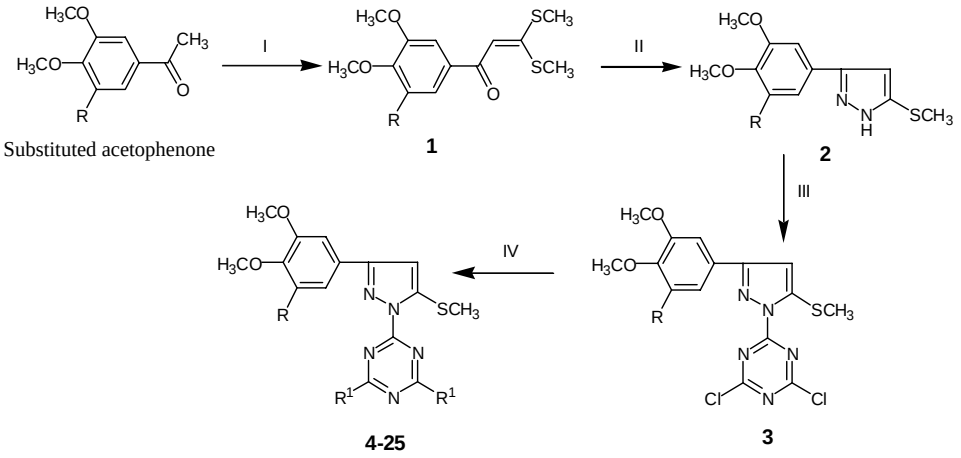


Scheme 1. Reagents and conditions: (I) CS_2, NaH, MeI, THF, 0 °C-rt (II) $NH_2NH_2{\cdot}H_2O$, MeOH, reflux. (III) Cyanuric chloride, K_2CO_3, THF, reflux. (IV) Various amines, K_2CO_3, THF, reflux.

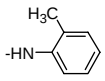
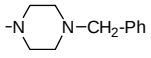
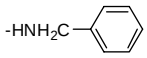
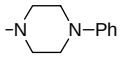
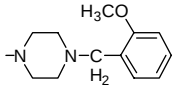
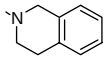
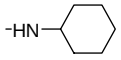
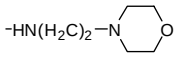
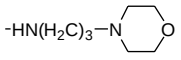
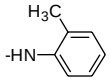
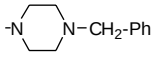
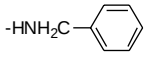
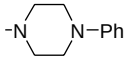
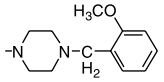
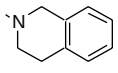
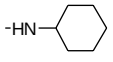
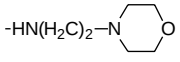
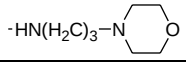
methyl iodide.[10,11] The compound **1** was reacted with hydrazine hydrate[12,13] in methanol to obtain 5-methylsulfanyl-3-(substituted-phenyl)-1*H*-pyrazole (**2**). The compound **2** was reacted with cyanuric chloride (2,4,6-trichloro-1,3,5-triazine) in the presence of K_2CO_3 to obtain 2,4-dichloro-6-[5-methylsulfanyl-3-(substituted-phenyl)-pyrazol-1-yl]-[1,3,5]-triazine (**3**), which was subjected to nucleophilic substitution with different amines to afford the final targeted compounds **4**–**25** (Table 1). All the synthesized compounds were well characterized by spectroscopic methods such as IR, mass, NMR, and elemental analysis.[17]

The in vitro antimalarial assay was carried out in 96-well microtiter plates according to the micro assay of Rieckmann.[14] The culture of *P. falciparum* NF-54 strain is routinely maintained in RPMI-1640 medium supplemented with 25 mM Hepes, 1% D-glucose, 0.23% sodium bicarbonate, and 10% heat-inactivated human serum.[15] The asynchronous parasite of *P. falciparum* was synchronized after 5% D-sorbitol treatment to obtain parasitized cells harboring only the ring stage.[16] For carrying out the assay, an initial ring stage parasitemia of ≈1% at 3% hematocrit in a total volume of 200 μL of RPMI-1640 medium 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 was 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 the presence of different concentrations of compounds. The tested concentration that inhibits the complete maturation into schizonts was recorded as the minimum inhibitory concentration (MIC). Cycloguanil was used as the standard reference drug.

Activities of all the tested compounds are shown in Table 1.

All the synthesized compounds **4**–**25** were evaluated for their antimalarial activity against *P. falciparum* strain NF-54 and the activity results are described in Table 1. Out of the 22 screened compounds, 6 compounds (**4**, **7**, **12**, **13**, **16**, and **24**) have shown MIC in the range between 1 and 2 μg/mL concentrations and 6 compounds (**14**, **15**, **17**, **19**, **20**, and **25**) have shown MIC of 10 μg/mL. The compound **4** has 3,4,5-trimethoxy phenyl group at position 3 of the pyrazole ring and *O*-tolyl amino group at positions 4 and 6 of the triazine ring, while the compound **15** having the same substituents at positions 4 and 6 of the triazine ring and 3,4-dimethoxy phenyl group at position 3 of the pyrazole ring reduced the activity from 1 to 10 μg/mL. The in vitro antimalarial result suggested that the *O*-tolyl amino group at positions 4 and 6 of the triazine ring and 3,4,5-trimethoxy phenyl substituent at position 3 of the pyrazole ring is very important in exhibiting antimalarial activity. The compound **5** showed MIC of 50 μg/mL having the 3,4,5-trimethoxy phenyl substituent at position 3 of the pyrazole ring and *N*-benzyl piperazino group at positions 4 and 6 of the triazine ring, while the compound **16** has shown MIC of 2 μg/mL having the same groups at positions 4 and 6 of the triazine nucleus and 3,4-dimethoxy phenyl group at position 3 of the pyrazole ring. The compound **7**, having 3,4,5-trimethoxy phenyl substituent at position 3 of the pyrazole ring and benzyl amino group at positions 4 and 6 of the triazine ring, has shown MIC of 2 μg/ mL, while the compound **18** showed MIC of >50 μg/mL having benzyl amino group at positions 4 and 6 of the triazine ring and 3,4-dimethoxy phenyl group at position 3 of the pyrazole ring; this indicates that the presence of 3,4-dimethoxy group at position 3 of the pyrazole ring, activity was reduced. The compound **12** showed MIC of 2 μg/mL, while the compound **23** showed MIC of 50 μg/mL, both having the cyclohexyl amino group at positions 4 and 6 of the triazine ring and different substituents at position 3 of the pyrazole ring. Compound **12** has 3,4,5-trimethoxy phenyl and compound **23** has 3,4-dimethoxy phenyl group at position 3 of the pyrazole ring. The compounds **13** and **14** have shown MIC

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

Compound	R	R^1	MIC (μg/mL)
4	OCH_3		1
5	OCH_3		50
6	OCH_3		>50
7	OCH_3		2
8	OCH_3		50
9	OCH_3		>50
10	OCH_3		50
11	OCH_3		>50
12	OCH_3		2
13	OCH_3		1
14	OCH_3		10
15	H		10
16	H		2
17	H		10
18	H		>50
19	H		10
20	H		10
21	H		50
22	H		50
23	H		>50
24	H		2
25	H		10

MIC, minimum inhibiting concentration for the development of ring stage parasite into the schizont stage during 40 h incubation.
MIC of cycloguanil = 64 μg/mL.

of 1 and 10 μg/mL, respectively. Both have 3,4,5-trimethoxy phenyl substituent at position 3 of the pyrazole ring and ethyl amino morpholino and propyl amino morpholino groups, respectively, at positions 4 and 6 of the triazine ring. Compounds **24** and **25** having 3,4-dimethoxy phenyl group at position 3 of the pyrazole ring and ethyl amino morpholino and propyl amino morpholino groups, respectively, at positions 4 and 6 of the triazine ring have shown MIC of 2 and 10 μg/mL, respectively. The antimalarial activity results have suggested that increase in the chain length of the appropriate amines decreases the activity of compounds.

SAR results indicate that the 3,4,5-trimethoxy phenyl and 3,4-dimethoxy phenyl groups at position 3 of the pyrazole ring and *O*-tolyl amino, benzyl amino, cyclohexyl amino *N*-ethyl amino morpholine, and *N*-benzyl piperazino groups at positions 4 and 6 of the triazine ring are crucial for antimalarial activity.

A series of 22 compounds were synthesized and screened against *P. falciparum* NF-54 strain. Of the screened compounds, 6 compounds have shown MIC in the range between 1 and 2 μg/mL. These compounds are 32 times more potent than the cycloguanil which was used as standard drug. These molecules can be useful for further lead optimization work in malaria chemotherapy.

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17. Spectroscopic data for compound **4** Yield: 65%; mp 196–198 °C; FAB-MS: 570 (M+1); IR (KBr): 3365, 3065, 2935, 2828, 1578, 1509, 1428, 1382 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ (ppm) 7.81 (d, 2H, J = 6.80 Hz, Ar-H), 7.40–7.10 (m, 8H, Ar-H), 6.37 (s, 1H, pyrazole-H), 3.99 (s, 6H, OCH_3), 3.92 (s, 3H, OCH_3), 2.52 (s, 3H, SCH_3), 2.32 (s, 6H, Ar-CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 165.66, 162.95, 154.75, 153.19, 151.66, 149.49, 146.31, 139.44, 136.24, 130.90, 126.77, 125.36, 111.23, 104.66, 103.72, 61.55, 56.48, 18.59, 18.12. Anal. Calcd for $C_{30}H_{31}N_7O_3S$: C, 63.25; H, 5.48; N, 17.21. Found: C, 63.15; H, 5.57; N, 17.45%; spectroscopic data for compound **5**. Yield: 61%; mp 190–192 °C; FAB-MS: 707 (M+1); IR (KBr): 3031, 2933, 2803, 1621, 1578, 1501, 1419, 1370 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.35–7.26 (m, 10H, Ar-H), 7.11 (s, 2H, Ar-H), 6.37 (s, 1H, pyrazole-H), 4.01–3.85 (m, 8H, NCH_2), 3.92 (s, 6H, OCH_3), 3.87 (s, 3H, OCH_3), 3.55 (s, 4H, NCH_2), 2.50–2.39 (m, 11H, NCH_2, SCH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 165.44, 162.92, 154.30, 153.88, 151.68, 145.42, 139.33, 129.88, 127.98, 120.69, 117.55, 104.32, 103.56, 61.23, 57.37, 56.42, 49.86, 44.06, 18.45. Anal. Calcd for $C_{38}H_{45}N_9O_3S$: C, 64.47; H, 6.41; N, 17.81. Found: C, 64.73; H, 6.55; N, 17.67%; Spectroscopic data for compound **15**. Yield: 65%; mp 190–192 °C; FAB-MS: 540 (M+1); IR (KBr): 3311, 3035, 2961, 2833, 1588, 1505, 1434, 1404, 1375 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.80 (d, 2H, J = 6.80 Hz, Ar-H), 7.58 (d, 1H, J = 1.68 Hz, Ar-H), 7.38 (dd, 1H, J = 1.66, 8.28 Hz, Ar-H), 7.25–7.10 (m, 6H, Ar-H), 6.90 (d, 1H, J = 8.30 Hz, Ar-H), 6.37 (s, 1H, pyrazole-H), 3.99 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 2.43 (s, 3H, SCH_3), 2.33 (s, 6H, Ar-CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 165.66, 162.95, 153.82, 154.75, 150.26, 149.39, 144.72, 146.42, 136.24, 139.41, 130.90, 126.78, 125.36, 119.78, 111.23, 109.76, 103.72, 56.48, 56.30, 18.59, 18.12. Anal. Calcd for $C_{29}H_{29}N_7O_2S$: C, 64.54; H, 5.42; N, 18.17. Found: C, 64.45; H, 5.65; N, 18.24%; Spectroscopic data for compound **16**. Yield: 62%; mp 195–198 °C; FAB-MS: 678 (M+1); IR (KBr): 3015, 2936, 2810, 1592, 1501, 1396, 1354 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.52 (d,1H, J = 1.80 Hz, Ar-H), 7.50–7.28 (m, 11H, Ar-H), 6.88 (d, 1H, J = 8.30 Hz, Ar-H), 6.40 (s, 1H, pyrazole-H), 3.95 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.90–3.82 (m, 8H, NCH_2), 3.55 (s, 4H, NCH_2), 2.50–2.42 (m, 11H, SCH_3, NCH_2); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 166.28, 162.15, 154.12, 150.09, 149.59, 145.58, 139.69, 128.85, 128.26, 127.52, 125.71, 119.49, 111.75, 109.85, 103.66, 56.23, 56.68, 44.28, 46.97, 45.85, 18.32. Anal. Calcd for $C_{37}H_{43}N_9O_2S$: C, 65.56; H, 6.39; N, 18.60. Found: C, 65.45; H, 6.56; N, 18.72%.