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Synthesis of substituted indole derivatives as a new class of antimalarial agents

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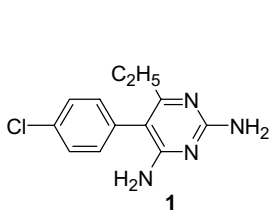
Abstract—A series of substituted indole derivatives were synthesized and evaluated for their in vitro antimalarial activity against *P. falciparum*. Out of the 24 compounds synthesized six compounds have shown MIC of 1 μg/mL. These compounds are in vitro several folds more active than pyrimethamine.

1. Introduction

Malaria remains the most serious and refractory health hazard amongst the parasitic diseases, affecting around 500 million people and causing around 2 million deaths annually.[1] According to WHO estimates 40% of the world's population presently lives under malarial threat. Due to the fact that the parasites are developing resistance to conventional antimalarial drugs, the development of drugs attacking crucial targets in the metabolism of the malaria pathogen is imperative.[2] Development of active and selective chemotherapeutic agents could be achieved by rational drug design taking into consideration the biochemical machinery of the parasite. One of the targets for drugs against malaria is the enzyme dihydrofolate reductase (DHFR). Pyrimethamine (**1**) is a specific inhibitor of the plasmodial DHFR. 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 indirectly implicated in the metabolism of amino acids and purine nucleotide. Inhibition of DHFR thus prevents biosynthesis of DNA leading to cell death.[3,4]

Indole and its derivatives represent one of the most active class of compounds possessing a wide spectrum of antiparasitic activities. Indole alkaloids as apicidin (**2**) have shown potent antimalarial activity against *P. falciparum*. Apicidin is known to be a potent inhibitor of HDAC (histone deacetylase), a nuclear enzyme that regulates gene transcription and the assembly of newly synthesized chromatin. Apicidin reversibly induces histone hyperacetylation, causing altered transcriptional regulation and ultimately cell death. Studies on apicidin have suggested that the indole region is a key constituent of enzyme binding and HDAC activity.[5] Compounds, which act on more than one target sites are more liable to be active. Keeping in view all of the above facts we have synthesized hybrid derivatives containing both indole (HDAC inhibitor) and pyrimidine moiety (DHFR inhibitor).



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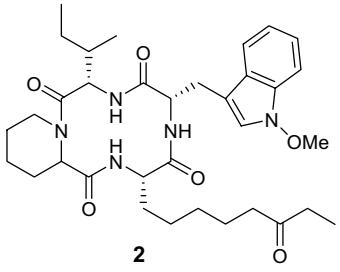

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Antimalarial drugs as quinine, mefloquine have piperidine nucleus and amopyroquine, cycloquine have pyrrolidine moiety. A large number of compounds having piperidine, pyrrolidine, piperazine and morpholine

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moiety have shown potent antimalarial activity.[6] These results prompted us to synthesize compounds having indole moiety along with pyrimidine and these cyclic amines at second position of pyrimidine ring.

As part of our ongoing programme devoted to the synthesis of diverse heterocycles as antiinfective agents,[7] we had previously reported antimalarial activity in substituted triazines, pyrimidines and quinolines.[8] This communication describes the synthesis of substituted indoles **2**–**5**(**a**–**f**) as antimalarial agents.

2. Chemistry

The chalcones **1**(**a**–**f**) were synthesized by reacting 3-formyl indole with different substituted acetophenones (**a**–**f**) by refluxing them in methanol in the presence of piperidine.[9] Cyclic carboxamidine hydrochlorides were synthesized by refluxing the corresponding cyclic amine with *S*-methyl-isothiourea sulfate in water according to a reported procedure.[10] The chalcones **1**(**a**–**f**) were further cyclized with imidine hydrochlorides in the presence of sodium isopropoxide (synthesized in situ by adding sodium metal in isopropanol) to afford the substituted indole derivatives **2**–**5**(**a**–**f**) 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 microtitre plates according to the micro assay of Rieckmann et al.[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% 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.

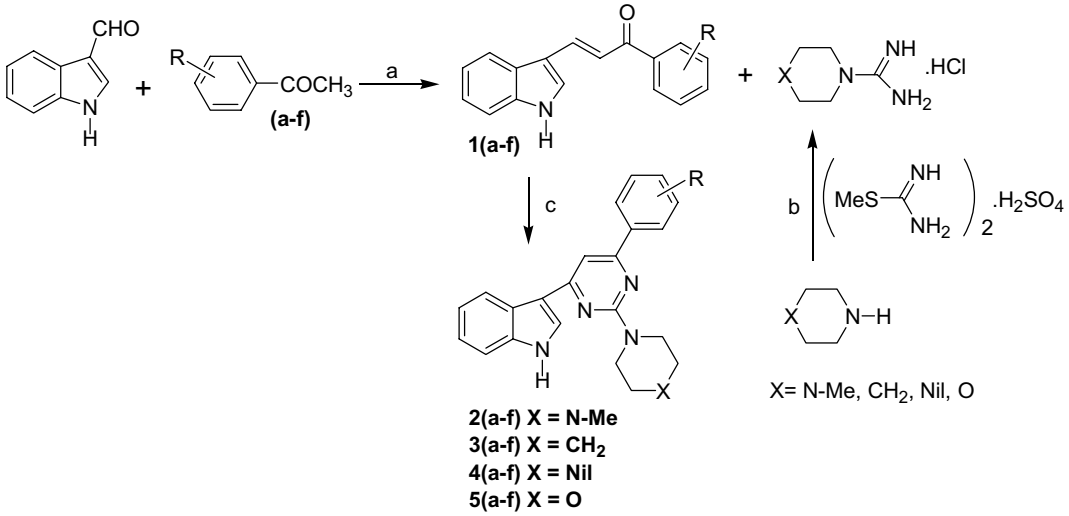
Table 1. Antimalarial in vitro activity against *P. falciparum*

S. No	R	MIC (μg/mL)			
		2(**a**–**e**) X = NMe	**3**(**a**–**e**) X = CH_2	**4**(**a**–**e**) X = nil	**5**(**a**–**e**) X = O
a	H	1	10	10	50
b	4-Me	1	10	10	50
c	4-OCH_3	1	10	10	50
d	3,4-Di-OCH_3	1	10	10	50
e	2,5-Di-OCH_3	1	10	10	50
f	4-Cl	1	10	10	50

MIC = Minimum inhibiting concentration for the development of ring stage parasite into the schizont stage during 40 h incubation. Standard drug, pyrimethamine, MIC 10 μg/mL.

4. Results and discussion

Among all the 24 compounds **2**–**5**(**a**–**f**) tested, six compounds showed MIC of 1 μg/mL, while 12 compounds



Scheme 1. Reagents and conditions: (a) Different acetophenone (**a**–**f**), piperidine, methanol, reflux, 8 h; (b) (i) different secondary amines, *S*-methylisothiourea sulfate, water, reflux, 15 min; (ii) barium chloride, reflux, 15 min; (c) different imidines, HCl, sodium isopropoxide, isopropanol, reflux, 8 h.

have shown MIC of 10 μg/mL. All the compounds, which had *N*-methyl piperazine group at 2-position of the pyrimidine ring showed a MIC of 1 μg/mL. Varying the substituents on the phenyl ring at the sixth position of the pyrimidine ring have no effect on the activity, while substitution at the second position plays a crucial role in exerting antimalarial activity. On replacing the piperazine ring with pyrrolidine and piperidine moiety the activity dropped to 10 μg/mL. On further substituting it with morpholine moiety the activity reduced further having a MIC of 50 μg/mL. These results emphasize the better efficacy of *N*-methyl piperazine group over pyrrolidine, piperidine and morpholine group in antimalarial activity.

5. Conclusion

Twenty-four substituted indole **2**–**5**(**a**–**f**) derivatives were synthesized as pyrimethamine analogues. Out of the synthesized compounds six compounds showed MIC of 1 μg/mL. These compounds are 10 times more potent than pyrimethamine. The present study suggested that the newly synthesized indole derivatives are new lead in antimalarial chemotherapy. These molecules can be very useful for further optimization work in malarial chemotherapy.

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14. Spectroscopic data for **2c**. MS: 400 (M+1); mp 130–132 °C; IR (KBr) 2932, 1645, 1574, 1484, 1319, 1280 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.70 (s, 1H, NH), 8.43 (d, 1H, $J = 8.6$ Hz), 8.09 (d, 2H, $J = 8.8$ Hz), 7.90 (s, 1H), 7.42 (d, 1H, $J = 8.2$ Hz), 7.29–7.26 (m, 2H), 7.27 (s, 1H), 7.01 (d, 2H, $J = 8.8$ Hz), 4.08 (t, 4H, $J = 4.9$ Hz), 2.58 (t, 4H, $J = 4.9$ Hz), 2.38 (s, 3H, NMe); ^{13}C ($CDCl_3$, 50 MHz): δ 168.3, 167.5, 166.5, 166.2, 142.6, 135.9, 133.5, 132.4, 130.7, 127.4, 126.7, 125.9, 120.5, 119.1, 117.2, 106.2, 55.8, 55.5, 46.7, 44.2. Anal. Calcd for $C_{24}H_{25}N_5O$: Calcd C, 72.16; H, 6.31; N, 17.53. Found: C, 72.52; H, 6.54; N, 17.71. Spectroscopic data for **2f**. MS : 404 (M+1); mp 204–206 °C; IR (KBr) 2956, 1654, 1568, 1478, 1325, 1275 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.83 (s, 1H, NH), 8.42 (d, 1H, $J = 8.1$ Hz), 8.05 (d, 2H, $J = 8.5$ Hz), 7.90 (s, 1H), 7.48 (d, 2H, $J = 8.5$ Hz), 7.41 (d, 1H, $J = 8.3$ Hz), 7.30 (s, 1H), 7.28–7.25 (m, 2H), 4.10 (t, 4H, $J = 5.4$ Hz), 2.57 (t, 4H, $J = 5.4$ Hz), 2.38 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz): 165.8, 163.6, 162.5, 162.3, 137.8, 135.9, 128.8, 128.7, 127.5, 125.9, 122.6, 122.0, 121.2, 115.9, 112.3, 104.5,

55.5, 46.7, 44.2. Anal. Calcd for $C_{23}H_{22}ClN_5$: Calcd C, 68.39; H, 5.49; N, 17.34. Found: C, 68.58; H, 5.68; N, 17.61. Spectroscopic data for **3b**. MS: 369 (M+1); mp decomposes at 240 °C; IR (KBr) 2924, 1648, 1576, 1486, 1328, 1284 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.9 (s, 1H, NH), 8.48 (d, 1H, $J = 8.2$ Hz), 8.03 (d, 2H, $J = 8.1$ Hz), 7.99 (s, 1H), 7.47 (d, 1H, $J = 8.3$ Hz), 7.34 (d, 2H, $J = 8.1$ Hz), 7.27 (s, 1H), 7.25–7.21 (m, 3H), 4.00 (t, 4H, $J = 4.2$ Hz), 1.70–1.65 (m, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 167.4, 167.1, 166.1, 142.5, 137.3, 135.6, 133.2, 132.6, 133.3, 131.7, 129.8, 126.7, 125.6, 120.5, 118.7, 103.9, 45.5, 26.1, 25.6, 18.9. Anal. Calcd for $C_{24}H_{24}N_4$: Calcd C, 78.23; H, 6.57; N, 15.21. Found C, 78.46; H, 6.72; N, 15.39. Spectroscopic data for **3f**. MS 389 (M+1); mp 228–230 °C; IR (KBr) 2926, 1638, 1580, 1480, 1325, 1272 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.60 (s, 1H, NH), 8.47 (d, 1H, $J = 8.2$ Hz), 8.05 (d, 2H, $J = 8.6$ Hz), 7.89 (s, 1H), 7.46 (d, 2H, $J = 8.6$ Hz), 7.39 (d, 1H, $J = 8.3$ Hz), 7.30 (s, 1H), 7.28–7.24 (m, 2H), 4.00 (t, 4H, $J = 4.2$ Hz), 1.71–1.65 (m, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 165.9, 163.7, 162.7, 162.6, 137.7, 135.9, 128.9, 128.6, 127.5, 125.9, 122.6, 122.0, 121.2, 115.9, 112.3, 100.9, 45.4, 26.0, 25.4. Anal. Calcd for $C_{23}H_{21}ClN_4$: Calcd C, 71.03; H, 5.44; N, 14.41. Found: C, 71.34; H, 5.65; N, 14.58. Spectroscopic data for **4a**. MS: 341 (M+1); mp 232–234 °C; IR (KBr) 2932, 1645, 1574, 1484, 1319, 1280 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.72 (s, 1H, NH), 8.58 (d, 1H, $J = 8.1$ Hz), 8.16 (d, 2H, $J = 8.4$ Hz), 8.05 (s, 1H), 7.54 (d, 1H, $J = 7.8$ Hz), 7.49–7.45 (m, 3H), 7.33 (s, 1H), 7.21–7.17 (m, 2H), 3.75 (t, 4H, $J = 5.2$ Hz), 2.04 (t, 4H, $J = 5.2$ Hz); ^{13}C ($CDCl_3$, 50 MHz): 168.3, 167.5, 166.5, 142.6, 135.9, 133.5, 132.4, 130.2, 129.4, 128.2, 126.3, 126.7, 125.9, 120.5, 119.1, 106.2, 43.8, 25.4. Anal. Calcd for $C_{22}H_{20}N_4$: Calcd C, 77.62; H, 5.92; N, 16.46. Found: C, 77.46; H, 5.77; N, 16.31. Spectroscopic data for **5d**. MS 417 (M+1); mp 220–222 °C; IR (KBr) 2948, 1636, 1584, 1486, 1325, 1265 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.62 (s, 1H, NH), 8.44 (d, 1H, $J = 8.2$ Hz), 7.95 (s, 1H), 7.73 (d, 1H, $J = 7.9$ Hz), 7.67 (s, 1H), 7.45 (d, 1H, $J = 8.4$ Hz), 7.32 (s, 1H), 7.26–7.21 (m, 2H), 6.97 (d, 1H, $J = 8.4$ Hz), 4.05 (t, 4H, $J = 4.2$ Hz), 4.00 (s, 3H, OMe), 3.97 (s, 3H, OMe), 3.87 (t, 4H, $J = 4.2$ Hz); ^{13}C NMR ($CDCl_3$, 50 MHz): 164.1, 163.2, 162.8, 151.4, 149.5, 137.4, 131.7, 126.7, 125.9, 123.3, 122.2, 121.7, 120.5, 116.9, 111.9, 111.5, 110.6, 102.2, 67.5, 56.5, 56.4, 45.1. Anal. Calcd for $C_{24}H_{24}N_4O_3$: Calcd C, 69.21; H, 5.81; N, 13.45. Found: C, 69.47; H, 5.74; N, 13.29. Spectroscopic data for **5e**. MS 417 (M+1); mp 236–238 °C; IR (KBr) 2936, 1642, 1578, 1488, 1324, 1284 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.68 (s, 1H, NH), 8.40 (d, 1H, $J = 8.1$ Hz), 7.94 (s, 1H), 7.68 (s, 1H), 7.43 (d, 1H, $J = 8.2$ Hz), 7.33 (s, 1H), 7.24 (m, 2H), 6.96 (d, 2H, $J = 7.8$ Hz), 4.00 (t, 4H, $J = 4.6$ Hz), 3.88 (s, 3H, OMe), 3.84 (s, 3H, OMe), 3.80 (t, 4H, $J = 4.6$ Hz); ^{13}C ($CDCl_3$, 50 MHz): δ 164.3, 163.3, 162.7, 151.5, 149.6, 137.5, 131.7, 126.7, 125.9, 123.3, 122.2, 121.7, 120.5, 116.8, 111.9, 111.6, 110.6, 102.2, 67.6, 56.6, 56.4, 45.2. Anal. Calcd for $C_{24}H_{24}N_4O_3$: Calcd C, 69.21; H, 5.81; N, 13.45. Found: C, 69.38; H, 5.68; N, 13.62.