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Syntheses of 2,4,6-trisubstituted triazines as antimalarial agents

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Abstract—A series of 2,4,6-trisubstituted-1,3,5-triazines (**2a**–**s**) were synthesized and evaluated for their in vitro antimalarial activity against *P. falciparum*. Out of the 19 compounds synthesized eight compounds showed MIC in the range of 1–2 μg/mL. These compounds are in vitro several times more active than cycloguanil.

1. Introduction

Despite years of continual effort, malaria is still one of the most deadly diseases affecting third world countries claiming more than 2 million lives annually.[1–3] *Plasmodium falciparum*, the causative agent of the most malignant forms of malaria is a particularly resistant parasite, which is known to have high adaptability by mutation. This mutability makes quite likely the development of resistance to chemotherapies currently being introduced.[4] A major thrust in this initiative is the identification of new targets that are critical to the disease process or essential for the survival of the parasite. The dihydrofolate reductase (DHFR) domain of *P. falciparum* is one of the few well defined targets in malarial chemotherapy. The enzyme catalyzes the nicotinamide adenine dinucleotide phosphate (NADPH) dependent reduction of dihydrofolate to tetrahydrofolate. DHFR has received considerable attention as it is the target of cycloguanil (**1**) and other antifolates used for prophylaxis and treatment of *P. falciparum* infection.[5] The rapid emergence of antifolate resistance in *P. falciparum* has unfortunately compromised the clinical use of the currently used drugs and thus highlights the urgent need for new effective antifolate antimalarials.[6,7] The design of novel chemical entities specially affecting selective parasitic folate metabolism could lead to better drugs for the treatment of malaria.

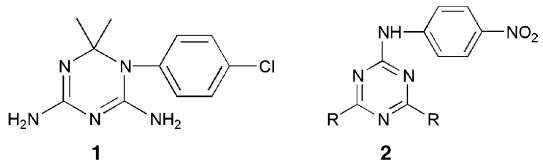

Figure 1.

2. Chemistry

The 2,4,6-trisubstituted-1,3,5-triazine compounds (**2**, Fig. 1) are synthesized from 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) by reacting them with different nucleophiles. The mono substituted triazine was synthesized by refluxing cyanuric chloride with *p*-nitro aniline in the presence of potassium carbonate in tetrahydrofuran (THF). Normally the first substitution in cyanuric chloride takes place at 0 °C but the amino group was highly deactivated due to the presence of nitro group at *para* position. The trisubstituted triazines (**2**) were synthesized by refluxing the monosubstituted triazine with different nucleophiles (R, Fig. 2) in the presence of potassium carbonate in THF. All the synthesized compounds were well characterized by spectroscopic data as IR, mass, NMR and elemental analysis.[11]

3. Biological activity

The in vitro antimalarial assay was carried out in 96-well microtitre plates according to the microassay of Rieckmann et al.[8] The culture of *P. falciparum* NF-54 strain was routinely maintained in medium RPMI-1640 supplemented with 25 mM HEPES, 1% D-glucose,

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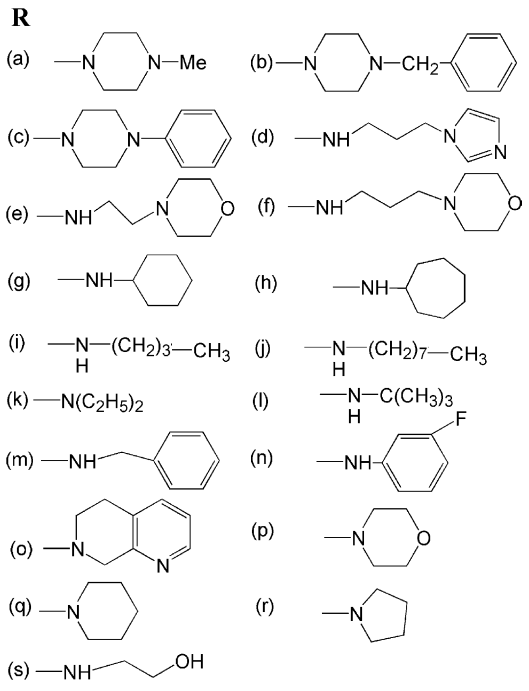

Figure 2.

0.23% sodium bicarbonate and 10% heat inactivated human serum.[9] The asynchronous parasite *P. falciparum* was synchronized after 5% D-Sorbitol treatment to obtain parasitized cells harbouring only the ring stage.[10] 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). Chloroquine and cycloguanil were 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*

Compd no.	MIC (μg/mL)	log *P*	pK_a
2a	1	0.228	1.88
2b	2	0.92	2.12
2c	10	1.64	2.86
2d	1	0.345	1.96
2e	2	−0.14	2.26
2f	2	−0.11	2.28
2g	1	0.356	2.58
2h	2	0.372	2.64
2i	2	0.396	2.32
2j	10	1.96	2.91
2k	10	2.06	3.05
2l	50	2.84	3.68
2m	10	1.68	2.88
2n	10	1.94	3.13
2o	10	1.64	2.94
2p	10	−0.06	2.98
2q	50	2.54	3.95
2r	50	2.46	3.62
2s	50	−0.56	2.86
1	64	2.94	3.82

MIC = minimum inhibiting concentration for the development of ring stage parasite into the schizont stage during 40 h incubation.
log *P* and pK_a values of all the compounds were calculated using ChemSilico software.

4. Results and discussion

Among all the compounds tested, eight compounds showed MIC in the range of 1–2 μg/mL whereas seven compounds showed a MIC of 10 μg/mL. The results showed a good structure–activity relationship. Results also showed good correlation with log *P* and pK_a values, which emphasize the importance of lipophilicity and basicity in the antimalarial activity of the synthesized compounds (**2a**–**s**). 1,3,5-Triazine having R as *N*-methyl piperazine **2a** showed a MIC of 1 μg/mL whereas when the methyl group was replaced with benzyl **2b** and phenyl group **2c** it showed a MIC of 2 μg/mL and 10 μg/mL, respectively. Compound **2a** has a log *P* value of 0.228 whereas compound **2b** and **2c** have shown log *P* values 0.92 and 1.64, respectively, which shows that activity decreases with increase in lipophilicity. Compound **2a**, **2b** and **2c** showed pK_a values of 1.88, 2.12 and 2.86, respectively, which shows that activity decreases with increasing pK_a or decreasing basicity of the compounds. Morpholine substituted compound **2p** showed a MIC of 10 μg/mL whereas when morpholine was replaced with 4-(3-aminopropyl) morpholine **2e** and 4-(2-aminoethyl) morpholine **2f** MIC reduced to 2 μg/mL. When the morpholine group in **2e** was replaced with imidazole **2d** the activity increased showing a MIC of 1 μg/mL. These results emphasize the role of short aliphatic chains and better efficacy of imidazole moiety over morpholine moiety in the antimalarial activity. In the compounds **2p**, **2e** and **2f** the same relation between lipophilicity and basicity with activity is observed. These compounds showed a negative log *P* value on introduction of oxygen atom in the molecule, its lipophilicity decreased to a greater amount but its basicity was not affected. These results emphasize the importance of both lipophilicity and basicity in the activity. With R as cyclohexylamine **2g** MIC was 1 μg/ml whereas slightly increasing the ring size to cycloheptylamine **2h** activity reduced slightly having a MIC of 2 μg/mL. In **2g** and **2h**, on increasing the ring size the lipophilicity and pK_a value increased slightly so the activity decreased. When the R group was *n*-butylamine **2i** it showed a MIC of 2 μg/mL whereas when chain length was increased to *n*-octylamine **2j** activity decreased having a MIC of 10 μg/mL. Branching in the alkyl chain also does not favour the activity. When the *n*-butylamine was replaced with diethylamine **2k** and *t*-butylamine **2l** it showed a decrease in activity having a MIC of 10 and 50 μg/mL.

Compounds **2i**, **2j**, **2k** and **2l** also followed the same relation of $\log P$ and pK_a values with activity. When the chain length was reduced to ethanolamine **2s** activity decreased further having a MIC of 50 μg/mL. In **2s** the $\log P$ value decreased extensively to −0.56 because of the introduction of polar hydroxyl group in the molecule whereas it showed pK_a value of 2.86. This molecule showed low activity in spite of the low $\log P$ value or low lypophilicity. This shows that some other factors such as basicity, etc., also contribute to the activity of the molecule. With R being cyclic secondary amines as piperidine **2q** and pyrrolidine **2r** it showed a MIC of 50 μg/mL. These results suggest that an unbranched aliphatic chain of medium size favours activity. When the chain length is increased or decreased above a particular size activity decreases.

5. Conclusion

The nineteen 2,4,6-trisubstituted-1,3,5-triazines (**2a**–**s**) were synthesized as cycloguanil analogues. Out of the synthesized compounds eight analogues have shown MIC in the range of 1–2 μg/mL, 32–64 times more potent than cycloguanil. These identified triazines can be new leads in antimalarial chemotherapy. These molecules are very useful for further optimization work in malarial chemotherapy.

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11. Spectroscopic data for **3a**: MS: 414 (M^{+1}), IR (KBr) 3420, 2932, 1571, 1484, 1319, 1280 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.18 (d, 2H, $J = 8.6$ Hz), 7.84 (d, 2H, $J = 8.6$ Hz), 3.85 (t, 2H, $J = 4.9$ Hz), 2.59 (t, 2H, $J = 4.9$ Hz), 2.34 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz): 170.1, 169.4, 152.3, 146.1, 129.9, 123.6, 59.9, 51.3, 48.2. Anal. Calcd for $C_{19}H_{27}N_9O_2$: Calculated C: 55.19, H: 6.58, N: 30.49, O: 7.74. Found: C: 55.31, H: 6.82, N: 30.38, O: 7.49; **3d**: MS 464 (M^{+1}), IR (KBr) 3409, 2943, 1582, 1494, 1326 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.15 (d, 2H, $J = 8.7$ Hz), 7.91 (d, 2H, $J = 8.7$ Hz), 7.58 (s, 2H), 7.47 (d, 2H, $J = 4.9$ Hz), 7.04 (d, 2H, $J = 4.9$ Hz), 4.09 (t, 4H, $J = 5.11$ Hz), 3.37 (m, 4H), 2.17 (t, 4H, $J = 5.0$ Hz); ^{13}C ($CDCl_3$, 50 MHz): 171.3, 169.4, 152.9, 145.6, 142.7, 134.0, 129.9, 124.6, 123.7, 49.5, 42.8, 36.3. Anal. Calcd for $C_{21}H_{25}N_{11}O_2$: Calculated C: 54.42, H: 5.44, N: 33.24, O: 6.90. Found: C: 54.36, H: 5.52, N: 33.28, O: 6.84.