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1-Aryl-4,6-diamino-1,2-dihydrotriazine as antimalarial agent: a new synthetic route

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Abstract—Some novel derivatives of 1-aryl-4,6-diamino-1,2-dihydrotriazines have been synthesized using neat technology under microwaves. These were tested in vitro against both sensitive and resistant *Plasmodium falciparum* strains for antimalarial activity.

1. Introduction

Most of the tropical and subtropical countries including Africa are prone to malarial diseases. In spite of continuous efforts to control malaria, it remains a major threat owing to drug resistant parasite.[1] This has highlighted the urgent need for the discovery and development of novel antimalarial agents aimed at combating the emerging resistant parasite. 1-Aryl-4,6-diamino-1,2 dihydrotriazines (**4**) are potent inhibitors of *Plasmodium falciparum* dihydrofolate reductase,[2] one of a few defined drug targets for antimalarial therapy. In the course of continuing efforts to develop new antimalarial drugs, a variety of novel-1-aryl-4,6-diamino-1,2-dihydrotriazine derivatives were synthesized as antimalarial agent. In addition to antimalarial activity, 1-aryl-4,6-diamino-1,2-dihydrotriazines (**4**) also exhibit antivitamin,[3–5] antitumor,[6,7] anticoccidial,[8,9] anthelminthic,[8,10] and antibacterial[3] activities.

2. Chemistry[20]

Conventionally, 1-aryl-4,6-diamino-1,2-dihydrotriazine (**4**) had been synthesized by two major routes viz. three[11,12] and two component synthesis.[13,14] In recent years, the use of solvent free conditions coupled with microwave have got momentum because of high efficiency, eco-friendly nature, cost effectiveness, and ease of manipulation. Though neat reaction technology is one step ahead in the direction of solvent less synthesis wherein no solvent is required at any stage of reaction. In continuation of our earlier work,[15] neat reaction technology showed better energy usage with improved yields and limits the use of hazardous solvents. All these fall in the domain of Green Chemistry.[16] This prompted us to perform one pot neat synthesis of 1-aryl-4,6-diamino-1,2-dihydrotriazines (**4**) using microwaves. The one pot synthesis minimizes the yield and energy loss. This condensation reaction is best effected under anhydrous conditions. The formation of water during reaction resulted in incomplete condensation or no reaction at all. This incomplete condensation leads to the formation of aryl biguanide as a side product which prevent the formation of titled compound. Triethyl orthoacetate[17] or triethyl orthoformate[18] were used in the synthesis of 1-aryl-4,6-diamino-1,2-dihydrotriazine (**4**) as dehydrating agents in normal course of reaction but in neat reaction using microwave no dehydrating agent is required. It is possible to take advantage of microwave exposure to induce equilibrium shifting by evaporation of light polar molecules such as water.

Literature reveals that only aromatic aldehydes can be used successfully for 1-aryl-4,6-diamino-1,2-dihydrotriazine (**4**) in three component synthesis barring aliphatic aldehydes.[11] While in two step reaction aliphatic aldehydes reacts rapidly, though standard protocol using formaldehyde does not give the desired product. Dimethoxymethane is used as the formaldehyde[19]

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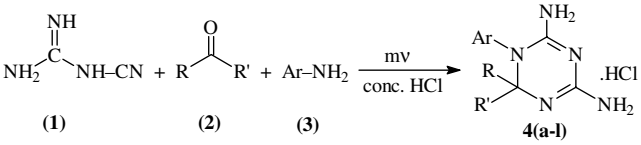
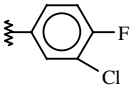
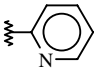
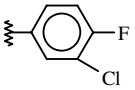
equivalent. However when formaldehyde is used under microwave, the desired product formed in excellent yields even in three component synthesis. Keeping in view it was thought worth while to synthesize 1-aryl-4,6-diamino-1,2-dihydrotriazines[22] (**4**) using one pot neat reaction technology under microwave.



4a	R′ = H	R = H	Ar = C_6H_5
4b	R′ = H	R = H	Ar = 
4c	R′ = H	R = H	Ar = C_6H_4–CH_3(*p*)
4d	R′ = H	R = H	Ar = C_6H_4–Cl(*p*)
4e	R′ = H	R = H	Ar = 
4f	R′ = H	R = C_6H_5	Ar = C_6H_5
4g	R′ = H	R = C_6H_5	Ar = 
4h	R′ = H	R = C_6H_5	Ar = C_6H_4–CH_3(*p*)
4i	R′ = H	R = C_6H_5	Ar = C_6H_4–Cl(*p*)
4j	R′ = H	R = C_6H_5	Ar = 
4k	R′ = CH_3	R = C_2H_5	Ar = C_6H_4–Cl(*p*)
4l	R′ = H	R = C_6H_4–OCH_3(*p*)	Ar = C_6H_5

3. Biological evaluation: antimalarial activity in vitro[21]

The antimalarial activity of compounds **4b**,**c**,**e**,**g**, and **4j** were tested against drug sensitive and resistant parasite lines. The effect of pyrimethamine and its analogues against both sensitive and resistant *P. falciparum* strain is shown in Table 1. Of the five new compounds, **4e** showed good in vitro antiplasmodial activity. Compounds **4g** and **4j** also exhibited some activity in both parasites, but **4e** found to be best among five, yet the activity of parent compound, Pyr* was at about 200-fold less concentration in case of sensitive and about 80-fold less concentration in resistant parasites. These compounds found equally active against both sensitive and resistant strains.

Table 1. Antimalarial activities, IC_{50}, and IC_{90} of new dihydrotriazine compounds against cycloguanil sensitive (FJB-D9) and resistant (FJB-D4) *P. falciparum* strain

Compound	Inhibitory activity (μM)			
	FJB-D9		FJB-D4	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}
4b	618.1	>1000	629.1	>1000
4c	584.5	>1000	501.04	>1000
4e	17.28	47.6	14.7	49.5
4g	81	388.4	52.9	363.6
4j	87.6	331.9	81.9	282.5
Pyr*	0.0016	0.006	0.005	0.028

*Pyr: pyrimethamine.

Acknowledgements

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20. *Experimental: general procedure*: A mixture of equimolar neat reactants in an Erlenmeyer flask, that is, (0.01 mol) aldehyde, (0.01 mol) cyanoguanidine, and (0.01 mol) aromatic amine with concd HCl was subjected to microwave irradiation for 2–3 min. Microwave irradiation was carried out in Kenstar Microwave Oven (2450 MHz, 800 W). More than 1 equiv of HCl was added as excess acid functions as a catalyst. Reaction was monitored at an interval of 30 s by TLC. Temperature of the reaction raise up to 80 °C in 30 s interval. The temperature of the reaction mixture was measured through a noncontact thermometer (AZ, Mini Gun type, model 8868). Final sticky solid obtained was triturated

with minimum amount of a suitable solvent to afford product in high yield. The structure of all the products were established on the basis of ^{1}H NMR, mass, and elemental analysis.

21. (a) *Biological activity in vitro*: Assay was conducted in pyrimethamine (Pyr) sensitive and resistant parasite lines. *P. falciparum* isolates, FJB-D9 (drug sensitive) and FJB-D4 (drug resistant) were cultured by the established method. FJB-D4 and FJB-D9 (Jabalpur, Central India) isolates were collected from patients in 1990. The growth medium used for culturing and drug sensitivity testing was RPMI 1640 with no added *p*-amino benzoic acid or folic acid (GIBCO, UK), supplemented with 25 mM HEPES buffer and sodium bicarbonate and enriched with 10% human AB^+ serum (obtained from blood donor belonging to malaria nonendemic area). Flat bottomed 96-well tissue culture plates were closed separately with various concentration of Pyr and five new compounds (0.0005–100 μg). An aliquot of 100 rmuL culture was added in control and drug dosed wells. The plates were incubated in candle jar at 37 °C for 30 h. After incubation, supernatant was aspirated from each well, pellet was mixed and thick/thin smears were prepared for microscopic examination. Growth of the parasites from duplicate wells of each and every concentrations of drug was monitored microscopically by counting number of Schizonts per 200 asexual parasites and total numbers of parasites per 500 RBC's. Schizonts having eight or more nuclei were counted. The assays were considered successful of 10% or more of the parasites in the control wells developed into schizonts with eight or more nuclei. Percent schizont maturation inhibition was calculated by the formula: $(1 - N_t/N_c) \times 100$, where N_t and N_c represent the number of schizont in the test and control wells respectively. Inhibitory concentrations 50 and 90 were calculated in both isolates by noting the drug concentrations at which 50% and 90% Schizont maturation were effected at 30 h; (b) Sundar, N.; Jacob, V. T.; Bhat, V. S.; Valecha, N.; Biswas, S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2269.
22. Compound **4a**: mp 230 °C (decomposition pt.); yield: 76%; ^{1}H NMR 60 MHz (DMSO): δ 4.6 (s, CH_2), 6.9 (br s, ex NH), 7.1–7.6 (m, Ar–C–H and NH_2), 7.80 (br s, ex NH), 8.58 (s, NH^+). Anal. Calcd for $C_9H_{12}ClN_5$: C, 47.89; H, 5.32; N, 31.04. Found: C, 47.84; H, 5.26; N, 30.86; *m/z* 190 $(M-Cl)^+$.
Compound **4b**: mp 222 °C (decomposition pt.); yield: 83.4%; ^{1}H NMR 60 MHz (DMSO): δ 4.67 (s, CH_2), 7.0–7.2 (m, Ar–C–H), 8.45 (m, Ar–CH). Anal. Calcd for $C_8H_{11}ClN_6$: C, 42.38; H, 4.85; N, 37.08. Found: C, 42.39; H, 4.70; N, 37.23; *m/z* 191 $(M-Cl)^+$.
Compound **4c**: mp 242–248 °C; yield: 93%; ^{1}H NMR 60 MHz (CD_3OD): δ 2.45 (s, Me-4′), 4.71 (s, CH_2), 7.3 (m, Ar–C–H), 7.8 (br s, NH). Anal. Calcd for $C_{10}H_{14}ClN_5$: C, 50.10; H, 5.84; N, 29.22. Found: C, 50.40; H, 5.84; N, 29.10; *m/z* 204 $(M-Cl)^+$.
Compound **4d**: mp 276–280 °C; yield: 95.6%; ^{1}H NMR 60 MHz (CD_3OD): δ 4.6 (s, CH_2), 7.3–7.7 (m, Ar–C–H). Anal. Calcd for $C_9H_{11}Cl_2N_5$: C, 41.54; H, 4.23; N, 26.92. Found: C, 41.38; H, 4.23; N, 26.80; *m/z* 224 $(M-Cl)^+$.
Compound **4e**: mp 184–188 °C; yield: 96%; ^{1}H NMR 300 MHz (DMSO): δ 4.79 (s, CH_2), 7.0 (br s, ex NH), 7.51 (m, Ar–C–H), 7.63 (s, NH_2), 7.75 (br s, ex NH), 8.78 (s, NH^+). Anal. Calcd for $C_9H_{10}Cl_2FN_5$: C, 38.84; H, 3.59; N, 25.18. Found: C, 38.52; H, 3.70; N, 25.42; *m/z* 242 $(M-Cl)^+$.
Compound **4f**: mp 215–220 °C; yield: 92.3%; ^{1}H NMR 60 MHz (DMSO): δ 6.0 (s, H-2), 7.4–7.6 (m, Ar–C–H). Anal. Calcd for $C_{15}H_{16}ClN_5$: C, 59.70; H, 5.31; N, 23.22. Found: C, 59.99; H, 5.53; N, 23.52; *m/z* 266 $(M-Cl)^+$.
Compound **4g**: mp 215–220 °C; yield: 84.3%; ^{1}H NMR 60 MHz (DMSO): δ 5.99 (s, H-2), 6.6–7.3 (m, Ar–C–H), 8.3 (m, Ar–C–H). Anal. Calcd for $C_{14}H_{15}ClN_6$: C, 55.54; H, 4.96; N, 27.77. Found: C, 55.29; H, 5.30; N, 27.52; *m/z* 267 $(M-Cl)^+$.
Compound **4h**: mp 225–230 °C; yield: 89.7%; ^{1}H NMR 60 MHz (CD_3OD): δ 2.4 (s, Me-4′), 6.02 (s, H-2), 7.4 (m, Ar–C–H and NH_2), 7.87 (br s, ex NH), 8.23 (s, NH^+). Anal. Calcd for $C_{16}H_{18}ClN_5$: C, 60.85; H, 5.70; N, 22.19. Found: C, 60.23; H, 5.72; N, 22.26; *m/z* 280 $(M-Cl)^+$.
Compound **4i**: mp 218–220 °C; yield: 89%; ^{1}H NMR 60 MHz (CD_3OD): δ 6.06 (s, H-2), 7.47 (m, Ar–C–H). Anal. Calcd for $C_{15}H_{15}Cl_2N_5$: C, 53.57; H, 4.46; N, 20.83. Found: C, 53.32; H, 4.47; N, 21.02; *m/z* 300 $(M-Cl)^+$.
Compound **4j**: mp 252 °C (decomposition pt.); yield: 78%; ^{1}H NMR 300 MHz (DMSO): δ 5.94 (s, H-2), 6.78 (br s, ex NH), 7.1 (m, Ar–C–H), 7.55 (m, Ar–C–H), 7.9 (br s, ex NH), 9.2 (s, NH^+). Anal. Calcd for $C_{15}H_{14}Cl_2FN_5$: C, 50.85; H, 3.95; N, 19.77. Found: C, 50.60; H, 3.58; N, 19.91; *m/z* 318 $(M-Cl)^+$.
Compound **4k**: mp 200–205 °C; yield: 68%; ^{1}H NMR 300 MHz (CD_3OD): δ 0.9 (t, CH_3), 1.28 (s, CH_3), 1.8 (q, CH_2), 7.28–7.35 (m, Ar–C–H). Anal. Calcd for $C_{12}H_{17}Cl_2N_5$: C, 47.68; H, 5.63; N, 23.18. Found: C, 47.72; H, 5.47; N, 23.28; *m/z* 266 $(M-Cl)^+$.
Compound **4l**: mp 176–180 °C; yield: 79%; ^{1}H NMR 300 MHz (DMSO): δ 3.83 (s, 4′-OCH_3), 5.87 (s, H-2), 6.8 (br s, ex NH), 7.27–7.49 (m, Ar–C–H and NH_2), 7.6–7.8 (m, Ar–C–H and NH), 8.9 (s, NH^+). Anal. Calcd for $C_{16}H_{18}ClN_5O$: C, 57.92; H, 5.43; N, 21.12. Found: C, 57.68; H, 5.71; N, 21.10; *m/z* 296 $(M-Cl)^+$.