

## SYNTHESIS OF NOVEL PHENAZINE DERIVATIVES BY SOLID STATE CHEMISTRY AND PRELIMINARY SCREENING AGAINST *PLASMODIUM FALCIPARUM* IN VITRO.

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### ABSTRACT

Fourteen novel phenazines, together with seven known phenazinic derivatives were synthesized through solid state chemistry by reaction of  $\beta$ -lapachone and eight other *o*-quinones with four different ortho-diamines, and screened against *Plasmodium falciparum* *in vitro*. The pyrido-phenazine derivatives showed the greatest suppression of parasitic growth while the 1,2-dichloro-derivatives exhibited the least activity for a given ortho-diamine. With respect to the ortho-quinones, the phenazines obtained from  $\beta$ -lapachone gave the best results.

**Keywords:** Phenazines, *o*-quinones, *o*-diamines, *Plasmodium falciparum*,  $\beta$ -lapachone

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### INTRODUCTION

Malaria is one of the most problematic parasitic infections of humans especially in sub-Saharan Africa. Due to its high morbidity and mortality, it is a threat to over two billion people living in areas of high incidence. *Plasmodium falciparum*, the causative agent of this malignant form of malaria, has high adaptability by mutation and is resistant to many types of anti-malarial drugs, which resistance is a serious setback to anti-malarial programmes since it precludes the use of cheap and previously effective drugs like chloroquine<sup>1</sup>. The search for efficient antimalarial drugs has led to an interest in phenazinic derivatives since natural and synthetic phenazines are known to have interesting biological activities<sup>2</sup>, including antimalarial<sup>3</sup>. A number of benzo[a]phenazines have been synthesized from 1,2-naphthoquinones, lapachol,  $\beta$ -lapachone and its derivatives, and shown to exhibit significant *in vitro* activities against *Plasmodium falciparum*<sup>1</sup>. To our knowledge, they were synthesized in solution according to the classical procedure described by Hooker, in concd CH<sub>3</sub>COOH between 4-6 hours<sup>4</sup>. The development of alternative environmentally friendly synthetic methods is strongly requested.

In the present work, a novel approach using solid state chemistry was used to synthesize various phenazines via Schiff base condensation of *o*-quinones and ortho-diamines under solvent free condition, and their antimalarial activities *in vitro* were tested against *Plasmodium falciparum*.

### EXPERIMENTAL

The phenazines were synthesised by reacting in closed test tubes under inert conditions (using argon or nitrogen gas), fine powders of ortho-diamines and ortho-quinones in a 2: 1 ratio at 35 °C for about 2-4 hours, during which the mixtures were agitated using an IKA-KS 130 basic reactor. In some cases one or two drops of acetic acid was added to moist the reaction medium. After the reaction, the product was

fixed with silica gel and eluted using hexane-ethyl acetate of increasing polarity to yield the different phenazines.

### Biological activity of phenazine derivatives

The compounds were each dissolved in a mixture of water/DMSO 0.02% (v/v)<sup>1</sup>, administered over a period of four days to the culture, and the number of parasites was determined daily. An untreated culture of plasmodia served as a reference.

#### (a) Culturing of *P. falciparum* NF54 strain

*P. falciparum* isolate NF54 and R strain were maintained in small Petri dishes (5 cm) according to a protocol from Moloney et al.<sup>9</sup> and Traeger and Williams<sup>10</sup> in a gaseous phase of 90 % N<sub>2</sub>, 5% CO<sub>2</sub> and 5% O<sub>2</sub>. Parasites were cultured in human erythrocytes (blood group A+) in RPMI640 medium (Sigma) supplemented with 25 μM HEPES, 20 mM sodium bicarbonate, and 10 % heat inactivated human A<sup>+</sup> plasma at 10 % (v/v) hematocrit. The parasitemia of infected erythrocytes was determined by light microscopy and estimated by Giemsa- stained smears. Parasitemias detected in the cultures were scored visually with a 100-fold oil immersion objective, counting at least 1000 infected erythrocytes to determine the parasitemia.

#### (b) Inhibitor experiments by monitoring multiplication and growth of plasmodia

Cultures were adjusted to a parasitemia of 0.5 %. Aliquots were diluted 1: 10 fold in RPMI-medium, dispensed into 12 -well microculture trays and incubated at 37 °C in a candle jar. Thereafter, growth medium was changed once a day for four days and inhibitors were added to the media in concentration of 20 μM as indicated. Parasitemias and stage distribution were estimated as triplicates daily from Giemsa stained smears by counting 1000 erythrocytes.

The compounds were administered over a period of four days to the culture and the number of parasites was determined daily. An untreated culture of plasmodia served as a reference. Each substance was tested in 4 different wells (1, 2, 3, 4; see table 2). Each substance was analysed over 4 days of in vitro treatment (days, 1, 2, 3, and 4). The averages were used for the graphic (see chart 1).

<sup>1</sup>H, 2D <sup>1</sup>H-<sup>1</sup>H COSY, <sup>13</sup>C, 2D HMQC and HMBC spectra were recorded with a Bruker Avance 500 MHz spectrometer. Chemical shifts are referenced to internal TMS (δ = 0) and coupling constants J are reported in Hz. Optical spectra were recorded with a NICOLET 510P FT-IR spectrometer, a UV-2101PC spectrometer, and Perkin-Elmer 241 polarimeter. The melting points (mp) were determined using a "BUCHI" B-540 instrument. Ortho-diamines were purchased from SIGMA.

### 11,12-dichloro-1,2-dihydro-3,3-dimethyl-3H-Benzo[a]pyrano[2,3-c]phenazine (1b)

Yellow needles from Hexane /AcOEt 10 %. Mp. 203 °C; <sup>1</sup>H-NMR: [500 MHz, CDCl<sub>3</sub>; J (Hz)] δ: 9.23 (dd, 1H, J = 7.5, 1.1 Hz, H-8), 8.34 (s, 1H, H-13), 8.3 (s, 1H, H-10), 8.30 (dd, 1H, J = 8.02, 1.4 Hz, H-5), 7.82-7.74 (m, 2H, H-6, H-7), 3.33 (t, 2H, J = 13.3, 6.6 Hz, H-1), 2.1 (t, 2H, J = 13.3, 6.6, Hz, H-2), 1.51 (s, 6H, -2CH<sub>3</sub>). <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>) δ: 26.8 (2CH<sub>3</sub>), 18.16 (CH<sub>2</sub>-1), 32.3 (CH<sub>2</sub>-2), 76.4(C-3), 152.6 (C-4a), 132 (C-4b), 122.3 (C-5), 130.1 (C-6), 127.9 (C-7), 125.2 (C-8), 130.2 (C-8a), 140.8 (8b), 145.2 (C-9a), 129.2 (C-10), 132.3 (C-11), 133.8 (C-12), 129.9 (C-13), 141.2 (C-13a), 138.7 (C-14a), 109 (C-14b); IR (CDCl<sub>3</sub>, cm<sup>-1</sup>) 3058, 2987, 2881, 1707, 1434, 1363, 1262, 1221, 1095, 892, 751, 700. UV (λ<sub>max</sub>, nm, log ε): 420.5(2.35), 370.5 (2.1), 308.5(5), 303.5(5), 296.5 (4.9), 293.5(4.8), 287.5 (4.85), 282 (4.4), 279.5 (3.9), 273 (3.6), 270.5(3.4), 265.5(1.6). 202.5 (2.1). EI-MS m/z: 383 (M<sup>+</sup>). HR-EIMS m/z: 382.06 Calcd. for C<sub>21</sub>H<sub>16</sub>Cl<sub>2</sub>N<sub>2</sub>O. Anal. for C<sub>21</sub>H<sub>16</sub>Cl<sub>2</sub>N<sub>2</sub>O (382.02): C 65.79; H 4.17; N 7.30. Found: C 64.52; H 3.89; N 6.25.

### 1,2-dihydro-3,3-dimethyl-3H-Benzo[a]pyrano[2,3-c]pyrido[3,2-b]quinoxaline (1c)

Deep yellow powder from Hexane /AcOEt 30 %. Mp. 192 °C. <sup>1</sup>H-NMR. [500 MHz, CDCl<sub>3</sub>; J (Hz)] δ: 9.30 (dd, 1H, J = 7.5, 1.5 Hz, H-8), 9.26 (dd, 1H, H-11), 8.7 (dd, 1H, J = 8.3, 1.5 Hz, H-13), 8.35 (dd, 1H, J = 7.5, 1.5 Hz, H-5), 7.86-7.76 (m, 2H, H-6, H-7), 7.74 (q, 1H, J = 8.3, 4.01 Hz, H-12), 3.43 (t, 2H, J = 13.3, 6.6, H-1), 2.15 (t, 2H, J = 13.3, 6.6, H-2), 1.6 (s, 6H, -2CH<sub>3</sub>). <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>) δ: 26 (2CH<sub>3</sub>), 18 (CH<sub>2</sub>-1), 32 (CH<sub>2</sub>-2), 78 (C-3), 153.8 (C-4a), 130 (C-4b), 122.4 (C-5), 128 (C-6), 130 (C-7), 125.3 (C-8), 131 (C-8a), 142 (8b), 150 (C-9a), 153.3 (C-11), 123.3 (C-12), 138.9 (C-13), 149.2 (C-

13a), 147 (C-14a), 109 (C-14b). IR (CDCl<sub>3</sub>, cm<sup>-1</sup>) 3053, 2982, 2841, 2304, 1717, 1419, 1368, 1262, 1221, 1095, 897, 741. UV (λ<sub>max</sub>, nm, log ε): 421.5(2.5), 357.5 (1.7), 308.5(4.7), 293 (5), 290 (4.9), 284.5 (4.9), 279.5 (4.4254.5 (2.2)). EI-MS *m/z*: 315 (M<sup>+</sup>). HR-EIMS gave molecular ion at *m/z*: 315.13 Calcd. for C<sub>20</sub>H<sub>17</sub>N<sub>3</sub>O. Anal. for C<sub>20</sub>H<sub>17</sub>N<sub>3</sub>O (315.13): C 76.19; H 5.39; N 13.33. Found: C 75.49; H 5.26; N 12.74.

**1,2-dihydro-3,3-dimethyl-3H-Benzo[a]pyrano[2,3-c]pyrimido[3,2-b]-2-mercapto-6-oxo-quinoxaline (1d)**

Yellow powder from Hex /AcOEt 30 %, Mp. 285 IR (CDCl<sub>3</sub>, cm<sup>-1</sup>), 3437.67, 2919.45, 2853.29, 2357.12, 2340.54, 1684.53, 1662.48, 1660.80. 1535.68, 1452.99, 1116.69, 1017.46 and 714.24. UV (λ<sub>max</sub>, nm, log ε): 457 (0.8), 318(2.95), 272 (1.40). <sup>1</sup>H-NMR. δ H [500 MHz, CDCl<sub>3</sub>; *J* (Hz)].... EI-MS *m/z*: 364 (M<sup>+</sup>) Calcd. for C<sub>19</sub>H<sub>16</sub>O<sub>2</sub>N<sub>4</sub>S.

**7,8-dichloro-1,3-dioxolo[4,5-b]phenazine (2b)**

Yellow powder from Hexane/AcOEt 10%. Mp. 328 °C. <sup>1</sup>H-NMR. [500 MHz, CDCl<sub>3</sub>; *J* (Hz)] δ: 8.3 (s, 2H, H-6, H-9), 7.4 (s, 2H, H-4, H-11) and 6.28 (s, 2H, H-2). <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>): δ: 102.5 (C-2), 154 (C-3a, C-11a), 104 (C-4, C-11), 143 (C-4a, C-10a), 142.5(C-5a, C-9a), 129 (C-6, C-9), 133 (C-7,8). IR (CDCl<sub>3</sub>, cm<sup>-1</sup>) 3053, 2988, 1422, 1264, 894, 737 and 704. (λ<sub>max</sub>, nm, log ε): 380(2.4), 376.5 (2.4), 352.5 (2.45), 281.5 (3.5), 276 (3) nm. EI-MS *m/z*: 293 (M<sup>+</sup>). HR-EIMS. *m/z*: 291.98 Calcd. for C<sub>13</sub>H<sub>6</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>.

**1,3-dioxolo[4,5-a]pyrido[3,2-b]quinoxaline (2c)**

Yellow powder from Hexane/AcOEt 60% Mp. 288 °C. <sup>1</sup>H-NMR. [500 MHz, CDCl<sub>3</sub>; *J* (Hz)] δ: 9.25 (dd, 1H, *J* = 3.8, 1.8 Hz, H-8), 8.52 (dd, 1H, *J* = 8.5, 1.8 Hz, H-6), 7.73 (q, 1H, *J* = 8.5, 3.8 Hz, H-7), 7.55 (s, 1H, H-4), 7.45 (s, 1H, H-11) and 6.3 (s, 1H, H-2). <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>) δ: 102.9 (C-2), 153.2 (C-3a), 153.1 (C-11a), 103.7 (C-4), 102.9 (C-11), 143.7 (C-4a), 145 (C-10a), 148.9(C-5a), 136.9(C-9a), 137.7 (C-6), 124.4 (C-7) 154.4 (C-8). UV (λ<sub>max</sub>, nm, log ε): 404.5 (2.6), 387.5 (2.6), 353.5(2.7), 355(2.75), 276 (2.85) and 218 (1.7) nm. EI-MS *m/z*: 225 (M<sup>+</sup>). HR-EIMS. *m/z*: 225.207 Calcd. for C<sub>12</sub>H<sub>7</sub>N<sub>3</sub>O<sub>2</sub>. Anal. for C<sub>12</sub>H<sub>7</sub>N<sub>3</sub>O<sub>2</sub> (225.2): C 64.00; H 13.13; N 18.66. Found: C 63.74; H 2.50; N 18.43.

**1,3-dioxolo[4,5-a]pyrimido[3,2-b] ]-2-mercapto-6-oxo-quinoxaline (2d)**

Yellow powder, from Hexane /AcOEt 60 %, Mp 255, IR (MeOH, cm<sup>-1</sup>), 3443.19, 2919.45, 2368.15, 1618.34, 1441.96, 1232.47, 1028.48, 863.09, 780.4 and 609.49. UV (λ<sub>max</sub>, nm, log ε): 408 (2.1), 303(5.0), 297 (4.80), 290(4.60), 287 (4.25) and 206 (1.85), EI-MS *m/z*: 274.13 (M<sup>+</sup>) Calcd. for C<sub>10</sub>H<sub>6</sub>N<sub>4</sub>O<sub>3</sub>S.

**2,3-dichloronaphtho[2,3-a]phenazine (3b)**

Yellow powder, Mp. 290 °C. <sup>1</sup>H-NMR. [500 MHz, CDCl<sub>3</sub>; *J* (Hz)] δ: 9.85 (s, 1H, H-13), 8.54 (s, 1H, H-4), 8.41 (s, 1H, H-1), 8.38 (s, 1H, H-8), 8.31 (dd, 1H, *J* = 10.5, 4.5 Hz, H-12), 8.15 (dd, 1H, *J* = 9.5, 4.5 Hz, H-9) 8.1 (d, 1H, *J* = 9.4 Hz, H-7), 7.8 (d, 1H, *J* = 9.4 Hz, H-6), 7.75-6.68 (m, 2H, H-10, H-11). <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>) δ: 129(C-1), 133 (C-2), 133 (C-3), 129 (C-4), 142 (C-4a), 143 (C-5a), 127 (C-6), 134 (C-7), 132 (C-7a), 127 (C-8), 132 (C-8a), 129 (C-9), 127 (C-10), 127 (C-11), 129 (C-12), 133 (C-12a), 125 (C-13), 130 (C-13a), 144 (13b), 167 (14a). IR (CDCl<sub>3</sub>, cm<sup>-1</sup>): 3053.7, 2988.52, 1422.36, 1264.65, 900.3, 737.16. UV (λ<sub>max</sub>, nm, log ε): 459.5 (1.9), 435 (1.7), 355 (2.1), 337.5 (2.8), 321(3.25), 316.5 (3.6), 311 (4.1), 307 (3.9), 283.5 (3.9), 279 (4.3), HR-EIMS (M<sup>+</sup>) *m/z* 348.02213 Calcd. for C<sub>20</sub>H<sub>10</sub>Cl<sub>2</sub>N<sub>2</sub>.

**Naphtho[2,3-a]pyrido[3,2-b]quinoxaline (3c):** Brownish-Yellow powder, Mp. 230 °C stereomeric mixture of 3c' and 3c''.

(3c'): <sup>1</sup>H-NMR. [500 MHz, CDCl<sub>3</sub>; *J* (Hz)] δ: 10.14 (s, 1H, H-13), 9.4-9.33 (m, 1H, H-2), 8.67 (dd, 1H, *J* = 8.5, 2 Hz, H-4), 8.42 (s, 1H, H-8), 8.32 (m, 1H, H-12), 8.16-8.13 (m, 2H, H-7, H-9), 7.83 (m, 2H, H-3, H-6) and 7.79-7.77 (m, 2H, H-10, H-11). <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>) δ: 155 (C-2), 125 (C-3), 138(C-4), 148 (C-4a), 143 (C-5a), 126 (C-6), 136 (C-7), 132 (C-7a), 127.5 (C-8), 132 (C-8a), 128 (C-9), 127.5 (C-10, C-11), 129 (C-12), 133 (C-12a), 127 (C-13), 130 (C-13a), 144 (C-13b) 150 (C-14a), HR-EIMS (M<sup>+</sup>) *m/z* 281.0953 Calcd. for C<sub>19</sub>H<sub>11</sub>N<sub>3</sub>.

(3c''): <sup>1</sup>H-NMR: [500 MHz, CDCl<sub>3</sub>; *J* (Hz)] δ: 9.9 (s, 1H, H-6), 9.4-9.33 (m, 1H, H-2), 8.8 (dd, 1H, H-4), 8.42 (s, 1H, H-11), 8.32-8.29 (m, 1H, H-7), 8.16-8.13 (m, 2H, H-12, H-10), 7.83-7.82 (m, 2H, H-3, H-13) and 7.79-7.70 (m, 2H, H-8, H-9). <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>) δ: 155 (C-2), 125 (C-3), 138(C-4),

148 (C-4a), 143 (C-5a), 126 (C-6), 129 (C-7), 27.5 (C-8, C-9), 128.5 (C-10), 132 (C-10a), 128 (C-11), 132 (C-11a), 136 (C-12), 126 (C-13), 140 (C-13a), 150 (C-14a), HR-EIMS ( $M^+$ )  $m/z$  281.0953 Calcd. for  $C_{19}H_{11}N_3$ .

**Naphtho[2,3-a]pyrimido[3,2-b]-2-mercapto-6-oxo-quinoxaline (3d)**

Orange-yellow powder from Hex /AcOEt 25 %, Mp. 295, IR ( $CDCl_3$ ,  $cm^{-1}$ ), 3454.21, 2924.96, 2853.29, 2362.62, 2335.07, 1656.97, 1546.71, 1508.12, 1452.99, 1116.69, 1034.00, 901.68 and 730.78. UV ( $\lambda_{max}$ , nm, log  $\epsilon$ ): 456 (0.2), 373(0.45), 353 (0.82), 335 (1.0), 294 (0.55) and 267 (0.95). EI-MS  $m/z$ : 330.13 ( $M^+$ ) Calcd. for  $C_{18}H_{10}N_4OS$ .

**Naphtho[2,1-a]phenanzine (4a)**

Yellow powder, Mp. 210 °C.  $^1H$ -NMR. [500 MHz,  $CDCl_3$ ;  $J$  (Hz)]  $\delta$ : 9.59 (d, 1H,  $J = 8.86$ , H-13), 9.05 (d, 1H,  $J = 9.5$ , H-7), 8.82 (d, 1H,  $J = 8.66$ , H-8), 8.46-8.45 (m, 1H, H-4), 8.37-8.35 (m, 1H, H-1), 8.32 (d, 1H,  $J = 9.5$ , H-6), 8.22 (d, 1H,  $J = 8.86$ , H-12), 8.11 (d, 1H,  $J = 8.0$ , H-11), 7.98-7.88 (m, 2H, H-2, H-3), 7.81 (td, 1H,  $J = 1.1$ , 8.0, 9.05, H-9) 7.75 (td, 1H, H-10).  $^{13}C$ -NMR (125 MHz,  $CDCl_3$ ):  $\delta$ : 129.4 (C-1), 130.2 (C-2), 130 (C-3), 129.94 (C-4), 142.5 (C-4a), 142.9 (C-5a), 127.37 (C-6), 127.45 (C-7), 130 (C-7a), 134 (C-7b), 123.3 (C-8), 127.0 (C-9), 127.0 (C-10), 128.9 (C-11), 130 (C-11a), 128.6 (C-12), 122.5 (C-13), 130 (13a), 142 (C-13b) and 142.5 (14a). IR ( $CDCl_3$ ,  $cm^{-1}$ ): 3441.81, 3059.23, 2983.97, 2356.97, 1430, 1265.51, 908.81, 744.95. UV ( $\lambda_{max}$ , nm, log  $\epsilon$ ): 390.5 (2.6), 358.5 (2.55), 312 (4.8), 290.5 (5.0), 287 (4.9), 284 (4.6), 276 (3.5). HR-EIMS ( $M^+$ )  $m/z$ : 280.10005 Calcd. for  $C_{20}H_{12}N_2$ .

**2,3-dichloronaphtho[2,1-a]phenanzine (4b)**

Yellow powder, Mp. 285 °C, slightly soluble in  $CH_2Cl_2/MeOH$ .  $^1H$ -NMR. [500 MHz,  $CDCl_3$ ;  $J$  (Hz)]  $\delta$ : 9.39 (d, 1H,  $J = 8.8$ , H-13), 9.03 (d, 1H,  $J = 9.6$ , H-7), 8.7 (d, 1H,  $J = 8.4$ , H-8), 8.53 (s, 1H, H-4), 8.46 (s, 1H, H-1), 8.18 (d, 1H,  $J = 9.6$ , H-6), 8.17 (d, 1H,  $J = 8.8$ , H-12), 8.05 (d, 1H,  $J = 7.8$ , H-11), 7.76-7.73 (m, 1H, H-10) and 7.71-7.68 (m, 1H, H-9).  $^{13}C$ -NMR identical to compound 4a except at  $\delta$ : 132.5 (C-2) and C-132 (3). HR-EIMS ( $M^+$ )  $m/z$  348.02210 Calcd. for  $C_{20}H_{10}Cl_2N_2$ .

**2,3-dichloro-5-hydroxybenzo[a]phenanzine (5b)**

Yellow powder, Mp. 288 °C, soluble in DMSO.  $^1H$ -NMR. [500 MHz,  $CDCl_3$ ;  $J$  (Hz)]  $\delta$ : 9.23 (dd, 1H, H-1), 8.6 (s, 1H, H-8), 8.4 (s, 1H, H-11), 8.35 (dd, 1H, H-4), 7.99-7.90 (m, 2H, H-2, H-3) and 7.2 (s, 1H, H-6). IR ( $CDCl_3$ ,  $cm^{-1}$ ): 3445.32, 3054.98, 2983.08, 2300.00, 1426.89, 1262.54, 897.89, and 743.81. HR-EIMS ( $M^+$ )  $m/z$  314.00133 Calcd. for  $C_{16}H_8Cl_2N_2O$ .

**6-methylnaphtho[2,3-a]phenanzine (6a)**

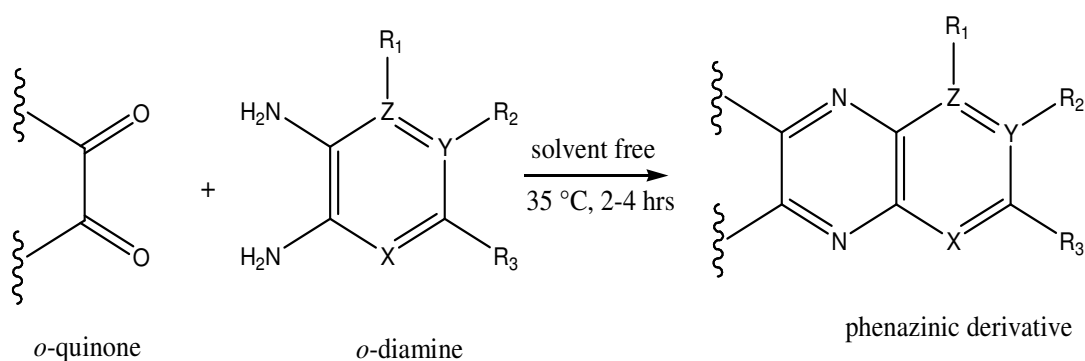
Yellow powder, Mp. 224 °C.  $^1H$ -NMR. [500 MHz,  $CDCl_3$ ;  $J$  (Hz)]  $\delta$ : 9.85 (s, 1H, H-13), 8.40 (dd, 1H,  $J = 6.9$ , 2.3, H-4), 8.33 (dd, 1H,  $J = 7.5$ , 2.4, H-1), 8.26 (dd, 1H,  $J = 6.88$ , 2.4, H-12), 8.22 (s, 1H, H-8), 8.08 (dd, 1H,  $J = 9.05$ , 2.3, H-9), 7.91-7.86 (m, 2H, H-2 and H-3), 7.85 (s, 1H, H-7), 7.66-7.60 (m, 2H, H-10 and H -11) and 2.86 (s, 1H,  $CH_3$ ).  $^{13}C$ -NMR  $\delta$ : (125 MHz,  $CDCl_3$ ): 129.4 (C-1), 129.5 (C-2), 129.5 (C-3), 129.3 (C-4), 142.2 (C-4a), 145 (C-5a), 134 (C-6), 132 (C-7), 130 (C-7a), 126 (C-8), 132 (C-8a), 127.9 (C-9), 127 (C-10), 127 (C-11), 129.2 (C-12), 134 (C-12a), 125.6 (C-13), 130 (C-13a), 143.8 (C-13b) and 141 (C-14a), 18.26 ( $CH_3$ ). IR ( $CDCl_3$ ,  $cm^{-1}$ ): 3461.63, 3053.78, 2977.64, 2308.76, 1422.36, 1270.09, 900.30, 737.16 and 704.53. UV ( $\lambda_{max}$ , nm, log  $\epsilon$ ): 439.5 (2.55), 421.5 (2.55), 417 (2.55), 357.5 (2.6), 345 (2.7), 319.5 (3.4), 311.5 (3.95), 300.5 (5.0), 299 (4.9), 292.5 (4.9), 288 (4.9), 281 (4.2), 274.5(2.9), 224.5 (2.1), 202.5 (2.1). HR-EIMS ( $M^+$ )  $m/z$  294.11568 Calcd. for  $C_{21}H_{14}N_2$ .

**2,3-dichloro-6-methyl naphtho[2,3-a]phenanzine (6b)**

Yellow powder, Mp. 260 °C.  $^1H$ -NMR. [500 MHz,  $CDCl_3$ ;  $J$  (Hz)]  $\delta$ : 9.81 (s, 1H, H-13), 8.5 (s, 1H, H-4), 8.46 (s, 1H, H-1), 8.29-8.25 (m, 1H, H-12), 8.25 (s, 1H, H-8), 8.11-8.06 (m, 1H, H-9), 7.9 (s, 1H, H-7), 7.69-7.63 (m, 2H, H-11 and H-12), 7.85 (s, 1H, H-7), 7.66-7.60 (m, 2H, H-10 and H -11) and 2.82 (s, 1H,  $CH_3$ ).  $^{13}C$ -NMR identical with that of compound 6a except at  $\delta$ : 132 for C-2 and C-3. IR ( $CDCl_3$ ,  $cm^{-1}$ ): 3467.7, 3059.21, 2977.64, 2874.32, 2308.76, 1618.13, 1427.79, 1264.65, 905.74 and 742.60. UV ( $\lambda_{max}$ , nm, log  $\epsilon$ ): 465 (2.51), 440 (2.51), 361 (2.6), 336 (2.7), 319.5 (3.35), 308 (5.0), 296.5 (5.0), 294 (5.0), 285 (5.0), 282.5 (4.55), 275.5 (4.2), 267.5 (2.5), 235.5 (2.35) and 208.5 (2.95). HR-EIMS ( $M^+$ )  $m/z$  262.03775 Calcd. for  $C_{21}H_{12}Cl_2N_2$ .

## RESULTS AND DISCUSSION

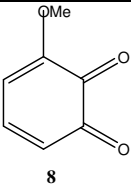
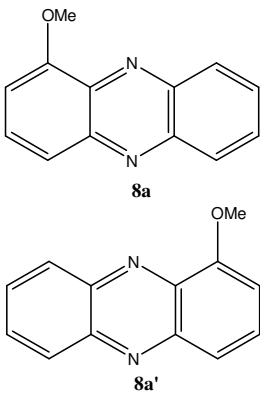
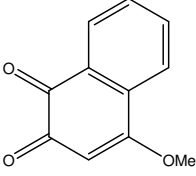
The solid state method employed consisted of heating a 2:1 mixture of fine powders of ortho-diamines and ortho-quinones in a closed test tube at 35 °C under inert conditions (using argon or nitrogen) for about 2-4 hours. Subsequently, the reaction mixture, fixed with silica gel, was eluted using hexane-ethyl acetate of increasing polarity to yield the different phenazines – thus, the method involves only a few steps unlike the classical procedure described by Hooker<sup>4</sup> for the synthesis of phenazines. Using this method, the various phenazines were synthesized from the reactions of  $\beta$ -lapachone and 8 other ortho-quinone derivatives<sup>5</sup> with 4 different heterocyclic ortho-diamines (Table 1). From ortho-phenylenediamine, the phenazines 1,2-dihydro-3,3-dimethyl-3H-benzo[a]pyrano[2,3-c]phenazine **1a**<sup>1</sup>, 1,3-dioxolo[4,5-b]phenazine **2a**<sup>6</sup>, naphtho[2,3-a]phenazine **3a**<sup>7</sup>, naphtho[2,1-a]phenazine **4a**, 5-hydroxybenzo[a]phenazine **5a**<sup>8</sup>, 6-methylnaphtho[2,3-a]phenazine **6a**, 1-phenazinol (isomers **7a** & **7a'**), 1-methoxyphenazine (isomers **8a** & **8a'**), and 5-methoxybenzo[a]phenazine (**9a**) (Table 1) were obtained; 4,5-dichloro-*o*-phenylenediamine gave 11,12-dichloro-1,2-dihydro-3,3-dimethyl-3H-Benzo[a]pyrano[2,3-c]phenazine **1b**, 7,8-dichloro-1,3-dioxolo[4,5-b]phenazine **2b**, 2,3-dichloronaphtho[2,3-a]phenazine **3b**, 2,3-dichloronaphtho[2,1-a]phenazine **4b**, 2,3-dichloro-5-hydroxybenzo[a]phenazine **5b** and 2,3-dichloro-6-methyl naphtho[2,3-a]phenazine **6b** (Table 1); 2,3-diaminopyridine gave 1,2-dihydro-3,3-dimethyl-3H-benzo[a]pyrano [2,3-c]pyrido[3,2-b]quinoxaline **1c**, 1,3-dioxolo[4,5-a]pyrido[3,2-b]quinoxaline **2c** and naphtho[2,3-a]pyrido[3,2-b]quinoxaline **3c** (mixture of 3C' & 3C'') (Table 1) while 4,5-diamino-6-hydroxy-2-mercaptopyrimidine gave 1,2-dihydro-3,3-dimethyl-3H-Benzo[a]pyrano[2,3-c]pyrimido[3,2-b]-2-mercapto-6-oxo-quinoxaline **1d**, 1,3-dioxolo[4,5-a]pyrimido[3,2-b] ]-2-mercapto-6-oxo-quinoxaline **2d** and naphtho[2,3-a]pyrimido[3,2-b]-2-mercapto-6-oxo-quinoxaline **3d** (Table 1). Of these phenazinic derivatives, compounds **1b**, **1c**, **1d**, **2b**, **2c**, **2d**, **3b**, **3c**, **3d**, **4a**, **4b**, **5b**, **6a**, and **6b** are reported for the first time. The derivatives **1b-6b** were synthesized in order to study the role of chlorine substituents in the *o*-phenylenediamine on their biological activity while the derivatives **1c-3c** and **1d-3d** were synthesized in order to investigate the influence of varying the structure of the ortho-diamine. Tables 1 also show that the derivatives **1c-3c** (from 2,3-diaminopyridine) were obtained in very high yields (90-95 %), whereas the yields of the other derivatives ranged from 32-70 %.



- a) *o*-phenylene diamine: X=Y=Z=C; R<sub>1</sub>=R<sub>2</sub>=R<sub>3</sub>=H;
- b) 4,5-dichloro-*o*-phenylene diamine: X=Y=Z=C; R<sub>1</sub>=H; R<sub>2</sub>=R<sub>3</sub>=Cl
- c) 2,3-diaminopyridine: X=N; Y=Z=C; R<sub>1</sub>=R<sub>2</sub>=R<sub>3</sub>=H
- d) 4,5-diamino-6-hydroxy-2-mercaptopyrimidine: X=Y=N; Z=C; R<sub>1</sub>=OH; R<sub>3</sub>=SH

Scheme-1



|   |   |     |
|---|---|-----|
|  <p style="text-align: center;"><b>8</b></p> | <p style="text-align: center;">8a and 8a'</p>  <p style="text-align: center;"><b>8a</b></p> <p style="text-align: center;"><b>8a'</b></p> | 60% |
|  <p style="text-align: center;"><b>9</b></p> | 9a  | 60% |

All the derivatives except compounds **1d**, **2d** and **3d** were tested *in vitro* against *Plasmodium falciparum*, and they showed considerable chemo-suppression of parasitic development (Table 2 and Chart 1 below). The pyrido-derivatives (**1c-3c**) showed the greatest suppression of parasitic growth while the 1,2-dichloro-derivatives (**1b-6b**) exhibited the least activity for a given ortho-diamine. Structurally, the pyrido-derivatives, with a nitrogen atom in the heterocyclic ring have some similarities with chloroquine, which is a well-known anti-malarial drug. With respect to the different ortho-quinones, the phenazines obtained using  $\beta$ -lapachone were found to give the best results.

Table-2: In vitro activity of synthesised phenazine against Plasmodium falciparum

|       | Reagent | Parasitemia in % in wells 1, 2, 3, 4 |      |      |      | Average |
|-------|---------|--------------------------------------|------|------|------|---------|
|       |         | 1                                    | 2    | 3    | 4    |         |
| Day 0 | Control | 0.53                                 | 0.58 | 0.55 | 0.55 | 0.55    |
| Day 1 | Control | 0.7                                  | 1.2  | 0.58 | 0.69 | 0.79    |
|       | 1a      | 0.82                                 | 0.74 | 0.56 | 0.9  | 0.75    |
|       | 1b      | 0.43                                 | 0.74 | 0.58 | 0.75 | 0.62    |
|       | 1c      | 0.83                                 | 0.53 | 0.65 | 0.5  | 0.62    |
|       | 2a      | 1.1                                  | 0.75 | 0.76 | 0.35 | 0.74    |
|       | 2b      | 0.68                                 | 0.72 | 0.63 | 0.8  | 0.7     |
|       | 2c      | 0.45                                 | 0.66 | 0.63 | 0.6  | 0.585   |
|       | 3a      | 0.3                                  | 0.25 | 0.84 | 0.4  | 0.447   |
|       | 3b      | 0.8                                  | 0.26 | 0.53 | 0.62 | 0.55    |
|       | 3c      | 0.45                                 | 0.66 | 0.63 | 0.6  | 0.585   |
|       | 4a      | 0.32                                 | 0.31 | 0.56 | 0.58 | 0.44    |
|       | 4b      | 0.28                                 | 0.5  | 0.49 | 0.82 | 0.52    |
|       | 5b      | 0.52                                 | 0.25 | 0.27 | 0.5  | 0.38    |
|       | 6a      | 0.44                                 | 0.68 | 0.52 | 0.45 | 0.52    |
|       | 6b      | 0.53                                 | 0.68 | 0.54 | 0.83 | 0.64    |
| 7a    | 0.5     | 0.33                                 | 0.42 | 0.45 | 0.42 |         |
| 7a'   | 0.38    | 0.7                                  | 0.4  | 0.4  | 0.47 |         |

|              |         |      |      |      |      |       |
|--------------|---------|------|------|------|------|-------|
|              | 8a      | 0.56 | 0.3  | 0.6  | 0.66 | 0.53  |
|              | 8a'     | 0.53 | 0.6  | 0.44 | 0.62 | 0.547 |
|              | 9a      | 0.4  | 0.83 | 0.62 | 0.53 | 0.595 |
| <b>Day 2</b> |         |      |      |      |      |       |
|              | Control | 1    | 1    | 0.7  | 1.4  | 1     |
|              | 1a      | 0.7  | 0.6  | 0.5  | 0.9  | 0.67  |
|              | 1b      | 0.6  | 0.72 | 0.67 | 0.73 | 0.68  |
|              | 1c      | 0.34 | 0.59 | 0.93 | 0.51 | 0.59  |
|              | 2a      | 1.1  | 0.57 | 0.7  | 1.0  | 0.84  |
|              | 2b      | 1.0  | 1.1  | 0.65 | 1.2  | 0.987 |
|              | 2c      | 0.47 | 0.69 | 1.1  | 1.0  | 0.81  |
|              | 3a      | 0.62 | 1.2  | 0.95 | 0.89 | 0.91  |
|              | 3b      | 1.2  | 1.4  | 1.2  | 1.4  | 1.3   |
|              | 3c      | 1.1  | 1.6  | 0.98 | 0.78 | 1.1   |
|              | 4a      | 0.77 | 0.85 | 1.1  | 0.7  | 0.855 |
|              | 4b      | 0.48 | 1.1  | 0.72 | 1.2  | 0.875 |
|              | 5b      | 1.4  | 1.1  | 0.6  | 0.36 | 0.865 |
|              | 6a      | 0.7  | 0.8  | 1.4  | 1.0  | 0.975 |
|              | 6b      | 0.37 | 1.4  | 0.78 | 0.78 | 0.83  |
|              | 7a      | 0.85 | 0.51 | 0.23 | 1.0  | 0.647 |
|              | 7a'     | 0.7  | 0.6  | 0.88 | 0.62 | 0.7   |
|              | 8a      | 0.79 | 0.96 | 0.72 | 0.7  | 0.79  |
|              | 8a'     | 0.82 | 0.73 | 0.9  | 0.47 | 0.81  |
|              | 9a      | 0.63 | 0.8  | 0.96 | 0.45 | 0.71  |
| <b>Day 3</b> |         |      |      |      |      |       |
|              | Control | 1.2  | 1.4  | 1.9  | 2.1  | 1.65  |
|              | 1a      | 1.1  | 1.2  | 1.4  | 1.5  | 1.3   |
|              | 1b      | 1.0  | 0.98 | 1.5  | 2.0  | 1.37  |
|              | 1c      | 0.4  | 0.84 | 1.2  | 0.6  | 0.76  |
|              | 2a      | 1.2  | 1.1  | 0.84 | 1.4  | 1.13  |
|              | 2b      | 1.1  | 1.8  | 1.4  | 1.0  | 1.32  |
|              | 2c      | 0.71 | 1.2  | 1.3  | 1.3  | 1.12  |
|              | 3a      | 0.75 | 1.4  | 1.5  | 1.2  | 1.2   |
|              | 3b      | 2.0  | 0.9  | 2.0  | 1.6  | 1.62  |
|              | 3c      | 1.6  | 1.6  | 0.7  | 0.82 | 1.18  |
|              | 4a      | 1.0  | 0.84 | 0.7  | 1.4  | 0.98  |
|              | 4b      | 1.4  | 1.1  | 0.94 | 1.3  | 1.18  |
|              | 5b      | 1.0  | 1.1  | 1.4  | 0.3  | 0.95  |
|              | 6a      | 0.7  | 1.7  | 1.3  | 1.4  | 1.27  |
|              | 6b      | 0.8  | 1.1  | 1.1  | 1.3  | 1.07  |
|              | 7a      | 0.9  | 1.3  | 0.9  | 1.7  | 1.2   |
|              | 7a'     | 1.3  | 0.85 | 1.0  | 1.1  | 1.06  |
|              | 8a      | 1.1  | 0.73 | 0.75 | 1.2  | 0.94  |
|              | 8a'     | 0.98 | 1.8  | 1.2  | 1.1  | 1.27  |
|              | 9a      | 1.0  | 0.85 | 1.1  | 1.4  | 1.08  |
| <b>Day 4</b> |         |      |      |      |      |       |
|              | Control | 1.9  | 2.0  | 2.2  | 2.6  | 2.17  |
|              | 1a      | 1.3  | 1.7  | 1.1  | 1.6  | 1.42  |
|              | 1b      | 2.1  | 1.6  | 2.2  | 1.5  | 1.85  |
|              | 1c      | 0.6  | 2.1  | 1.4  | 1.5  | 1.4   |
|              | 2a      | 1.5  | 2.3  | 2.2  | 2.2  | 2.0   |
|              | 2b      | 2.0  | 1.8  | 1.4  | 1.9  | 1.77  |

|     |     |      |     |     |      |
|-----|-----|------|-----|-----|------|
| 2c  | 1.6 | 1.2  | 1.9 | 2.0 | 1.67 |
| 3a  | 1.8 | 1.7  | 2.6 | 1.6 | 1.92 |
| 3b  | 2.2 | 1.6  | 2.2 | 1.7 | 1.92 |
| 3c  | 1.5 | 1.6  | 1.9 | 1.9 | 1.72 |
| 4a  | 2.3 | 1.5  | 1.3 | 2.4 | 1.87 |
| 4b  | 2.2 | 1.7  | 2.0 | 1.5 | 1.85 |
| 5b  | 1.5 | 1.0  | 0.7 | 0.8 | 1.0  |
| 6a  | 1.3 | 1.4  | 1.0 | 1.6 | 1.32 |
| 6b  | 1.6 | 1.6  | 1.6 | 2.0 | 1.7  |
| 7a  | 2.0 | 2.0  | 1.1 | 1.0 | 1.77 |
| 7a' | 1.8 | 2.6  | 2.6 | 2.0 | 2.25 |
| 8a  | 2.4 | 2.1  | 1.2 | 2.3 | 2.0  |
| 8a' | 1.9 | 2.2  | 1.9 | 2.5 | 2.12 |
| 9a  | 1.0 | 0.86 | 1.9 | 1.6 | 1.34 |

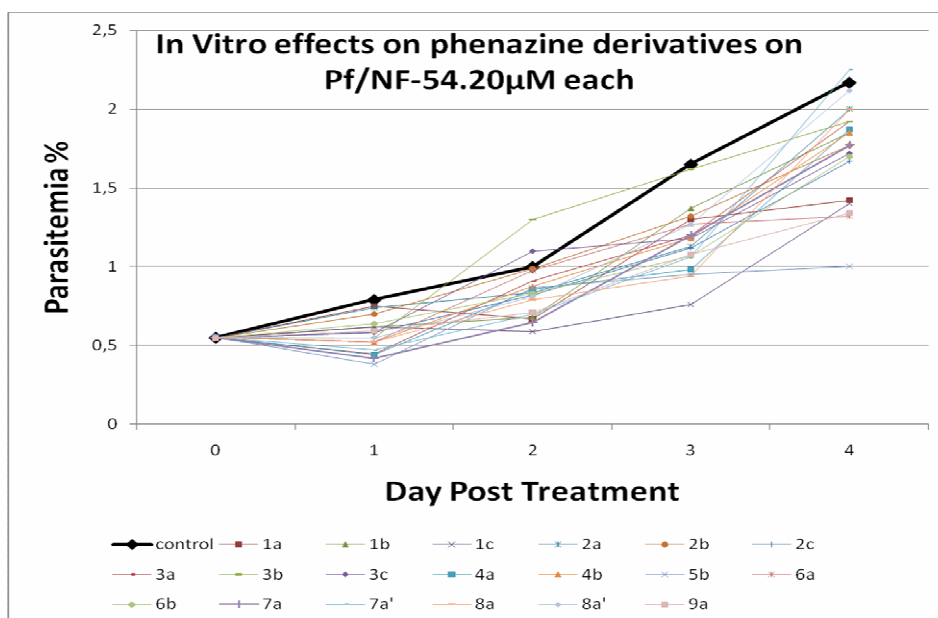


Fig.-1: *In vitro* effects of synthesised phenazines against *Plasmodium falciparum*.

### CONCLUSION

Novel phenazine derivatives were synthesis by reaction of ortho-diamines and ortho-quinones using solid state chemistry and screened against *plasmodium falciparum in vitro*. All the different phenazinic derivatives show considerable suppression of parasitic development with pyrido and  $\beta$ -lapachone derivatives being the most active. Structure activity relationship (SAR) could be established justifying the activity of  $\beta$ -lapachone and pyrido derivatives due to their pyrane and hetero nitrogen ring structures.

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