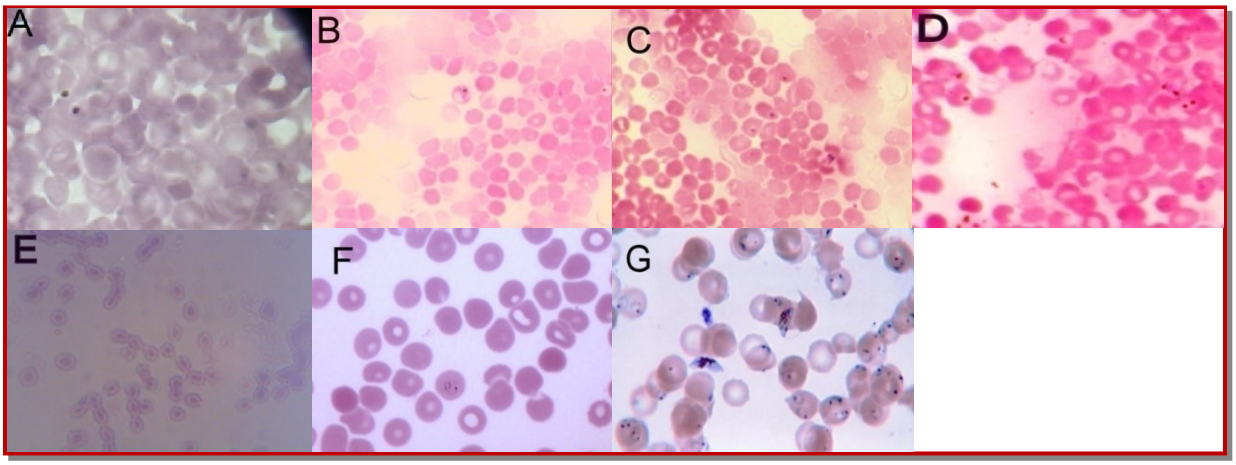

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Synthesis, characterization, and anti-plasmodial activity of 2,6-substituted benzothiazole derivatives

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Abstract

Six compounds of benzothiazole derivatives were synthesized, and structures were confirmed by FT-IR, ^{1}H-NMR, ^{13}C-NMR and LC-MS. The resulting compounds were evaluated for anti-plasmodial activity against *Plasmodium falciparum* by Giemsa stain. Among them the compounds **3b** and **4** showed potent anti-plasmodial activity against *P. falciparum*. The com-pounds **3a**, **5a**, **5b** also showed moderate anti-plasmodial activity. The structure-activity relationships of benzothiazole derivatives also discussed which were useful for exploring and developing benzothiazole derivatives as novel anti-malarial drugs.

Introduction

Malaria is one of the major causes of mortality around the world and is caused by protozoa *Plasmodium falciparum*. The emerging resistance to the existing anti-plasmodial drugs (Fidock, 2010), absence of newly found anti-plasmodial drugs (Ekland and Fidock, 2008) and poor development of clinically approved vaccines (Wisniewski et al., 2010) further compromise the effici-ent control of this disease.

Several anti-malarial drugs have been formulated for the treatment and prevention of the disease, but these have led to develop of resistance by the parasites to most of the drugs in use. Thus, there is a constant need for developing new anti-malarial compounds (Werns-dorfer, 1991).

Substituted quinolines are historically among the most important anti-plasmodial drugs and their use in the 20th century provided well-founded hopes for the eradication of malaria (Foley and Tilley, 1997). The important drugs of this family include chloroquine, quinine, mefloquine, amodiaquine, piperaquine and primaquine. However, the rapid spread of the malaria parasite resistance (Martin et al., 2009; Trape et al., 2011) to the above anti-malarial drugs alerted to the efforts to develop alternative anti-malarial drugs. Many heterocyclic compounds (Bekhit et al., 2012; Navarrete et al., 2001; Morgan et al., 2008; Guan et al., 2005) pyra-zole, benzimidazole, pyrimidines, guanides, indoline, quinazoline and benzothiazole (Burger and Sawhey, 1968) etc possess significant anti-plasmodial activity. Further, it has been reported that benzothiazole containing nitro group (Hout et al., 2004) exhibited potent anti-plasmodial activity against *P. falciparum*.

Hence, on the basis of literature review (Kumar et al., 2014), the present study involves the synthesis of different benzothiazole derivatives which are contain-ing nitro group. The resulting compounds were subjec-ted to anti-plasmodial activity against *P. falciparum*.

Materials and Methods

The synthetic starting material, reagents, and solvents were of analytical reagent grade or the highest quality commercially available. These were purchased from Sigma-Aldrich Chemical Co. and Merck Chemical Co.

The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in DMSO-d_6 solvent on Bruker 300 MHz spectrophotometer using tetramethylsilane as an internal reference. The apparent resonance multiplicity was described as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet) and m (multiplet). Infrared measurements were recorded in the range 400–4000 cm^{-1} by Perkin Elmer. Melting points were recorded by Labtronics digital melting point apparatus. Elemental analysis was carried out using Perkin Elmer CHNS. Mass spectra were recorded on a Thermo LCQ Deca XP MAX at 70 eV. Thin layer chromatography (TLC) analysis was carried out on 5 x 20 cm plate coated with silica gel GF_{254}.

Synthesis of tert-butyl 4-[(6-nitro-1,3-benzothiazol-2-L)carbamoyl]piperidine-1-carboxylate (2)

To a solution of N-boc-piperidine-4-carboxylic acid (10 g, 0.042 mol) in 400 mL THF, 2-amino-6-nitrobenzothiazole (19.1 g, 0.0984 mol), EDC. HCl (20.2 g, 0.106 mol) and HOBt (11.7, 0.082 mol) was added followed by triethylamine (57.5 mL, 0.041 mol) was added at 0°C. The resulting reaction mixture was allowed at room temperature and stirred for 20 hours. The reaction was monitored by TLC. The reaction mass was concentrated under reduced pressure, and separated between ethyl acetate (750 mL) and water (500 mL). The combined ethyl acetate layer was washed with 2N HCl (2 x 200 mL), 10% $NaHCO_3$ (2 x 200 mL), brine (1 x 400 mL) and dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure to afford compound **2**. Pale yellow solid; Yield 67%; mp 262-263°C; IR (KBr) ν_{max} in cm^{-1}: 3194 (NH, amide), 3083 (CH, aromatic), 2968, 2862 (CH, aliphatic), 1682 (C=O), 1324, 1521 (NO_2), 1442 (CH_2, bend), 1267 (C-N, amide), 1036, 901 (C-N), 747 (CH, bend), 686 (C-S); ^{1}H NMR (DMSO-d_6) δ ppm: 1.40 (9H), 1.41-1.46 (2H), 1.86 (2H), 2.76-2.80 (m, 4H), 3.98 (d, 2H), 7.87 (d, 1H, J=9.0 Hz), 8.26 (dd, 2H, J=2.4, 9.0 Hz), 9.0 (d, 1H, J=2.4 Hz), 12.8 (s, 1H); ^{13}C NMR (DMSO-d_6) δ ppm: 27.6, 28.1, 41.5, 78.7, 119.0, 120.58, 121.8, 132.2, 142.9, 153.4, 153.8, 163.5, 174.6; LC-MS (ESI) m/z : 405.7 $(M-H)^-$.

Synthesis of N-(6-nitro-1,3-benzothiazol-2-yL) piperidine-4-carboxamide HCl (3)

The above compound **2** (10 g, 0.020 mol) was dissolved in 60 mL 1,4-dioxane, to that saturated HCl in ether (90 mL) was added at 0°C. The reaction mixture was stirred for 2 hours at room temperature. The reaction completion was monitored by TLC. To the resulting residue, diethyl ether was added and the solid filtered off. The off-white solid was dried under vacuum to afford compound **3**. Yield 84%; mp 303-305°C; IR (KBr) ν_{max} in cm^{-1}: 3458 (NH)), 3091 (CH, aromatic), 2962, 2815 (CH, aliphatic), 1695 (C=O), 1341, 1522 (NO_2), 1448 (CH2, bend), 1276 (C-N, amide), 1037, 899 (C-N), 748 (CH, bend), 684 (C-S); ^{1}H NMR (DMSO-d_6) δ ppm: 1.81-1.93 (2H), 2.04 (2H), 2.90 (3H), 3.31 (2H), 7.89 (1H), 8.54 (dd, 1H, J=3,9 Hz), 9.0 (d, 1H, J=3 Hz), 9.21 (1H), 12.9 (1H); ^{13}C NMR (DMSO-d_6) δ ppm: 24.7, 42.3, 119.2, 120.8, 122.0, 132.4, 143.1, 153.6, 163.6, 174.13; LC-MS (ESI) m/z: 307.0 $(M+H)^+$.

Synthesis of 1-(3,5-difluorobenzoyl)-N-(6-nitro-1,3-benzothiazol-2-yL) piperidine-4-carboxamide (3a)

To a solution of compound **3** (0.4, 0.0015 mol) in 12 mL THF, 3,5-difluorobenzoic acid (0.184 g, 0.0011 mol), EDC. HCl (0.266 g, 0.0014 mol) and HOBt (0.189, 0.0014 mol) was added. Then reaction mixture was cooled to 0°C and triethylamine (0.73 mL, 0.0052 mol) was added. The resulting solution was allowed at room temperature and stirred for 14 hours. The reaction was monitored by TLC. The reaction mass was concentrated under reduced pressure and resulting crude product was separated between ethyl acetate (50 mL) and water (50 mL). The combined ethyl acetate layer was washed with 2N HCl (2 x 25 mL), 10% $NaHCO_3$ (2 x 25 mL), brine (1 x 50 mL) and dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure to afford compound **3a.** Off–white sold; Yield 27%; mp 279-281°C; IR (KBr) ν_{max} in cm^{-1}: 3434 (NH), 3089 (CH, aromatic), 2965, 2862 (CH, aliphatic), 1692 (C=O), 1616 (C=N), 1335, 1527 (NO_2), 1444 (CH, bend), 1265 (C-N, amide), 1039 (C-N, amine), 752 (CH, bend), 690 (C-S, stretch); ^{1}H NMR (DMSO-d_6) δ ppm: 1.68 (2H), 1.87 (1H), 2.01 (1H), 2.90 (2H), 3.15 (1H), 3.57 (1H), 4.47 (1H), 7.20 (d, 2H, J=6 Hz), 7.36 (t, 1H, J=9 Hz), 7.89 (d, 1H, J=9 Hz), 8.28 (dd, 1H, J=9, 2.4 Hz), 9.05 (d, 1H, J=2.4 Hz), 12.84 (1H); ^{13}C NMR (DMSO-d_6) δ ppm: 27.2, 41.4, 104.7, 110.1, 118.9, 120.5, 121.7, 132.1, 139.6, 142.9, 153.37, 160.5, 163.4, 166.2, 174.3; LC-MS (ESI) m/z: 445.2 $(M-H)^-$; Anal. calcd. for $C_{20}H_{16}F_2N_4O_4S$ (446)%: C, 53.81; H, 3.61; N, 12.55; Found: C, 53.88;H, 3.22; N,1 2.59.

Synthesis of 1-(2-furoyl)-N-(6-nitro-1,3-benzothiazol-2 -yL) piperidine-4-carboxamide (3b)

To a solution compound **3** (0.4 g, 0.019 mol) in 10 mL THF, triethylamine (0.56 mL, 0.0040 mol) was added at 0°C followed by 2-furoylchloride was added. The reaction mixture was allowed at room temperature, and stirred for 1 hour. The reaction was monitored by TLC. The reaction solution was concentrated under reduced pressure and separated between ethyl acetate (50 mL) and water (50 mL). The combined ethyl acetate layer was washed with 2N HCl (1 x 30 mL), 10% $NaHCO_3$ (1 x 40 mL), brine (1 x 150 mL) and dried over anhydrous Na_2SO_4. The ethyl acetate layer was concentrated under reduced pressure to afford compound **3b**. Yellow solid; Yield 39% mp 268-269°C; IR (KBr)ν_{max} in cm^{-1}: 3431 (NH), 3172 (CH, aromatic), 2958, 2859 (CH, aliphatic), 1694 (C=O), 1608 (C=N), 1335, 1527 (NO_2), 1445 (CH, bend), 1263 (C-N, amide), 1159 (C-O, furan), 1022, 904 (C-N, amine), 754 (CH, bend), 685 (C-S, stretch); ^{1}H NMR (DMSO-d_6) δ ppm: 1.61-1.68 (2H), 1.95-1.99 (2H),

2.88-2.95 (m, 3H), 4.35 (2H), 6.62 (dd, 1H, J=3.4, 1.7 Hz), 6.99 (d, 1H, J=3.4 Hz), 7.83-7.84 (m, 1H), 7.88 (d, 1H, J=8.9 Hz), 8.27 (dd, 1H, J=8.9, 2.4 Hz), 9.05 (d, 1H, J=2.4 Hz), 12.87 (s, 1H); ^{13}C NMR (DMSO-d_6) δ ppm: 28.5, 42.0, 111.7, 115.7, 119.4, 120.9, 122.1, 132.6, 143.5, 145.0, 147.53, 153.8, 158.8, 163.9, 174.8. LC-MS (ESI) m/z: 399.2 $(M-H)^-$; Anal. calcd. for $C_{18}H_{16}N_4O_5S$ (400)%: C, 53.99; H, 4.03; N, 13.99; Found: C, 53.37; H, 3.88; N, 13.45.

Synthesis of 1-[(2E)-3-(2-furyl)prop-2-enoyl]-N-(6-nitro-1,3-benzothiazol-2-yL) piperidine-4-carboxamide (3c)

Prepared as reported above for **3b** starting from compound **3** and (2E)-3-(furan-2-yl) acryloyl chloride. This reaction was carried out at room temperature for 2 hours. Yellow solid; Yield 50%; mp 283-284°C; IR (KBr) ν_{max} in cm^{-1}: 3427 (NH), 3156 (CH, Aromatic), 2953, 2862 (CH, aliphatic), 1688 (C=O), 1643 (C=C), 1585 (C=N), 1333, 1529 (NO_2), 1444 (CH, bend), 1262 (C-N, amide), 1160 (C-O, furan), 1020, 899 (C-N, amine), 746 (CH, bend); ^{1}H NMR (DMSO-d_6) δ ppm: 1.60 (2H), 1.97-2.0 (2H), 2.81-2.96 (2H), 3.20 (1H), 4.26 (1H), 4.49 (1H), 6.62 (dd, 1H, J=3.3, 1.8 Hz), 6.88 (d, 1H, J=3.3 Hz), 6.97 (d, 1H, J=15.2 Hz), 7.37 (d, 1H, J=15.2 Hz), 7.80 (s, 1H), 7.88 (d, 1H, J=9 Hz), 8.27 (dd, 1H, J=9, 2.4 Hz), 9.04 (d, 1H, J=2.4 Hz), 12.85 (s, 1H); ^{13}C NMR (DMSO-d_6) δ ppm: 29.0, 42.0, 112.9, 114.2, 115.6, 119.4, 120.9, 122.1, 129.2, 132.6, 143.3, 145.2, 151.7, 153.8, 163.9, 164.5, 174.9; LC-MS (ESI) m/z: 425 $(M-H)^-$; Anal. calcd. for $C_{20}H_{18}N_4O_5S$ (426)%: C, 56.33; H, 4.25; N, 13.14; Found: C, 56.88; H, 4.98; N, 13.32

Synthesis of 1-[(2,5-dichloro-3-thienyl)sulfonyl]-N-(6-nitro-1,3-benzothiazol-2-yL)piperidine-4- carboxamide (4)

To a solution compound **3** (0.35 g, 0.0102 mol) in 10 mL 1,2-dichloroethane, pyridine (0.42 mL, 0.0052 mol) and 2,5-dichlorothiophene-3-sulfonyl chloride (0.249 g, 0.00102 mol) was added. The reaction mixture was heated to 90°C for 10 hours. The reaction was monitored by TLC. Then resulting reaction mixture was cooled to room temperature, diluted with 30 mL of ethyl acetate. The combined ethyl acetate layer was washed with 2N HCl (1 x 25 mL), brine (1 x 40 mL) and dried over anhydrous Na_2SO_4. The ethyl acetate layer was concentrated under reduced pressure to afford compound **4**. Pale yellow solid; Yield 45%; mp 259-260° C; IR (KBr) ν_{max} in cm^{-1}: 3424 (NH), 2963, 2847 (CH), 1703 (C=O), 1609 (C=N), 1337, 1521 (NO_2), 1445 (CH, bend), 1386, 1164 (S=O), 1268 (C-N, amide), 1038 (C-N, amine), 717 (CH, bend), 635 (C-S, stretch); ^{1}H NMR (DMSO-d_6) δ ppm: 1.53-1.65 (2H), 1.84-1.96 (2H), 2.62-2.69 (3H), 3.68-3.72 (2H), 7.36 (s,1H), 7.82 (d,1H, J=9 Hz), 8.21 (dd,1H, J=9, 1.8 Hz), 8.98 (d,1H, J=1.8 Hz), 12.78 (s,1H); ^{13}C NMR (DMSO-d_6) δ ppm: 27.2, 44.9, 119.08, 120.6, 121.8, 126.7, 127.2, 130.0, 132.2, 133.1, 143.0, 153.4, 163.4, 174.2; LC-MS (ESI) m/z :519.1$(M-H)^-$ Anal. calcd. for $C_{17}H_{14}Cl_2N_4O_5S_3$ (520)%: C, 39.16; H, 2.71; N, 10.75; Found: C, 40.1; H, 2.88; N, 10.11.

Synthesis of N^1-(4-fluorophenyl)- N^4-(6-nitrobenzo[d] thiazol-2-yL)piperidine-1,4-dicarboxamide (5a)

To a solution compound **3** (0.6 g, 0.075 mol) in 10 mL THF, triethylamine was added at 0°C followed by 4-fluorophenylisocyate (0.22 g, 0.0074 mol) in 3 mL dichloromethane was added. The reaction mixture was allowed to room temperature and stirred for 4 hours. The reaction was monitored by TLC. The crude product was purified by flash column chromatography ($CHCl_3$/ MeOH 4%) to afford **5a.** Pale yellow solid; Yield 28%; mp 266-267°C; IR (KBr) ν_{max} in cm^{-1}: 3403 (NH, urea), 3349 (NH, amide), 3167, 3064 (CH, aromatic), 2962, 2853 (CH, aliphatic), 1693 (C=O), 1636 (C=O, urea), 1335, 1525 (NO_2), 1432 (CH, bend), 1265 (C-N, amide), 1237 (C-F), 1039, 903 (C-N, amine), 752 (CH, bend), 693 (C-S, Stretch); ^{1}H-NMR (DMSO-d_6) δ ppm: 1.59 (2H), 1.91 (2H), 2.83-2.90 (3H), 4.16 (2H), 7.07 (t,2H, J=9 Hz), 7.45 (d, 2H, J=9.0 Hz), 7.89 (d,1H, J=9.0 Hz), 8.33-8.23 (m,1H), 8.58 (s, 1H), 9.06 (d, 1H, J=2.4 Hz), 12.84 (s, 1H); ^{13}C NMR (DMSO-d_6) δ ppm: 28.2, 42.1, 43.6, 115.06, 115.35, 119.4, 120.9, 121.8, 122.2, 132.6, 137.3, 143.3, 153.9, 155.3, 163.9, 175.1; LC-MS (ESI) m/z: 442.0 $(M-H)^-$ Anal. calcd. for $C_{20}H_{18}FN_5O_4S$ (443)%: C,5 4.17; H, 4.09; N, 15.79; Found: C, 54.74; H, 4.33; N,15.45.

1-(p-tolylthiocarbamoyl)-N-(6-nitrobenzo[d]thiazol-2-yL) piperidine-4-carboxamide (5b)

Prepared as reported above for **5a**, starting from compound 3 and 4-methyl phenyl isothiocyanate. This reaction was carried out at room temperature for 3 hours. Pale yellow solid; Yield 58%; mp 263-264°C; IR (KBr) ν_{max} in cm^{-1}: 3383 (NH), 3173 (CH, Aromatic), 2923, 2859 (CH, aliphatic), 1689 (C=O), 1329, 1517 (NO_2), 1444 (CH, bend), 1266 (C-N, amide), 1033, 906 (C -N, amine), 748 (CH, bend); ^{1}H NMR (DMSO-d_6) δ ppm: 1.66-1.73 (2H), 1.95 (2H), 2.26 (3H), 2.93 (1H), 3.17 (2H), 4.74 (2H), 7.11 (q,4H, J=8.4 Hz), 7.89 (d,1H, J=8.9 Hz), 8.28 (dd, 1H, J=9,2.4 Hz), 9.06 (d, 1H, J=2.4 Hz), 9.25 (s, 1H), 12.84 (s, 1H); ^{13}C NMR (DMSO-d_6) δ ppm: 20.5, 27.6, 41.4, 47.4, 118.9, 120.5, 121.7, 125.4, 128.4, 132.1, 133.4, 138.4, 142.9, 153.4, 163.5, 174.5,181.2, LC-MS (ESI) m/z: 456.0 $(M+H)^+$ Anal. calcd. for $C_{21}H_{21}N_5O_3S_2$ (455)%: C, 55.37; H, 4.65; N, 15.37; Found: C, 55.78; H, 4.21; N, 15.23.

In vitro anti-plasmodial activity of benzothiazole derivatives

P. falciparum - parasite cultivation

The parasite culture *P. falciparum* was obtained from the Jawaharlal Nehru Centre for Advanced Scientific Research, Indian Institute of Science, Bangalore, India. Malarial parasites of *P. falciparum* were cultivated in human O Rh^+ red blood cells using RPMI 1640 medium containing 25 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (Sigma) supplemented

with O Rh+ serum (10%), 5% sodium bicarbonate and 40 µg/mL of gentamicin sulfate. Cultures were kept at 37°C under an atmosphere of 5% O_2, 3-5% CO_2 and N_2. Hematocrits were adjusted at 5% and parasite cultures were used when they exhibit 2% parasitemia (Trager, 1987).

Anti-plasmodial activity

The synthesized benzothiazole derivatives with different concentrations (100, 50, 25, 12.5, 6.25 and 3.125 µg/mL) were incorporated in 96 well tissue culture plate containing 200 µL of *P. falciparum* culture with fresh red blood cells diluted to 2% hematocrit. Negative control was maintained with fresh red blood cells and 2% parasitized *P. falciparum* diluted to 2% hematocrit and positive control was maintained with parasitized blood culture treated with chloroquine (Azas et al., 2002). Parasitemia was evaluated after 24, 48 hours of incubation by Giemsa stain and the average percentage suppression of parasitemia was calculated using following formula:

$$\%\text{Suppression} = \frac{\%\text{Parasitemia in control} - \%\text{Parasitemia in test}}{\%\text{Parasitemia in control}} \times 100$$

Assessment of chemical injury to erythrocytes

To assess any chemical injury to erythrocytes that might be attributed to the synthetic compounds, 200 µL of erythrocytes was incubated with 200 µg/mL of the all synthetic compounds at a dose equal to the high used in the anti-plasmodial assay. The conditions of the experiment were maintained as in the case of anti-plasmodial assay. After 48 hours of incubation, thin blood smears were stained with Giemsa stain and observed for the morphological changes under high-power light microscope. The morphological features observed of the treated erythrocytes were compared with the untreated erythrocytes (Waako et al., 2007).

Anti-plasmodial activity calculation and statistical analysis

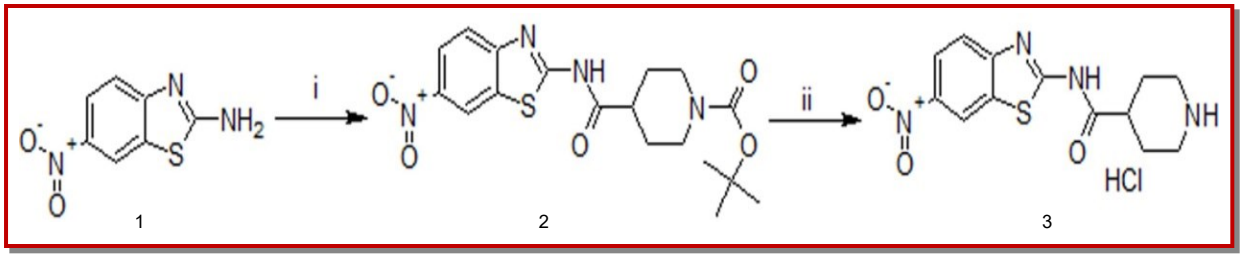
The anti-plasmodial activity of benzothiazole derivatives were expressed by the inhibitory concentration (IC_{50}) values that were calculated (concentration of synthetic compounds in the X-axis and percentage of inhibition by the synthetic compounds in the Y-axis) using office XP (SDAS) software. The anti-plasmodial activity was analyzed in accordance with the norms of Rasoanaivo et al., 1992.

Results

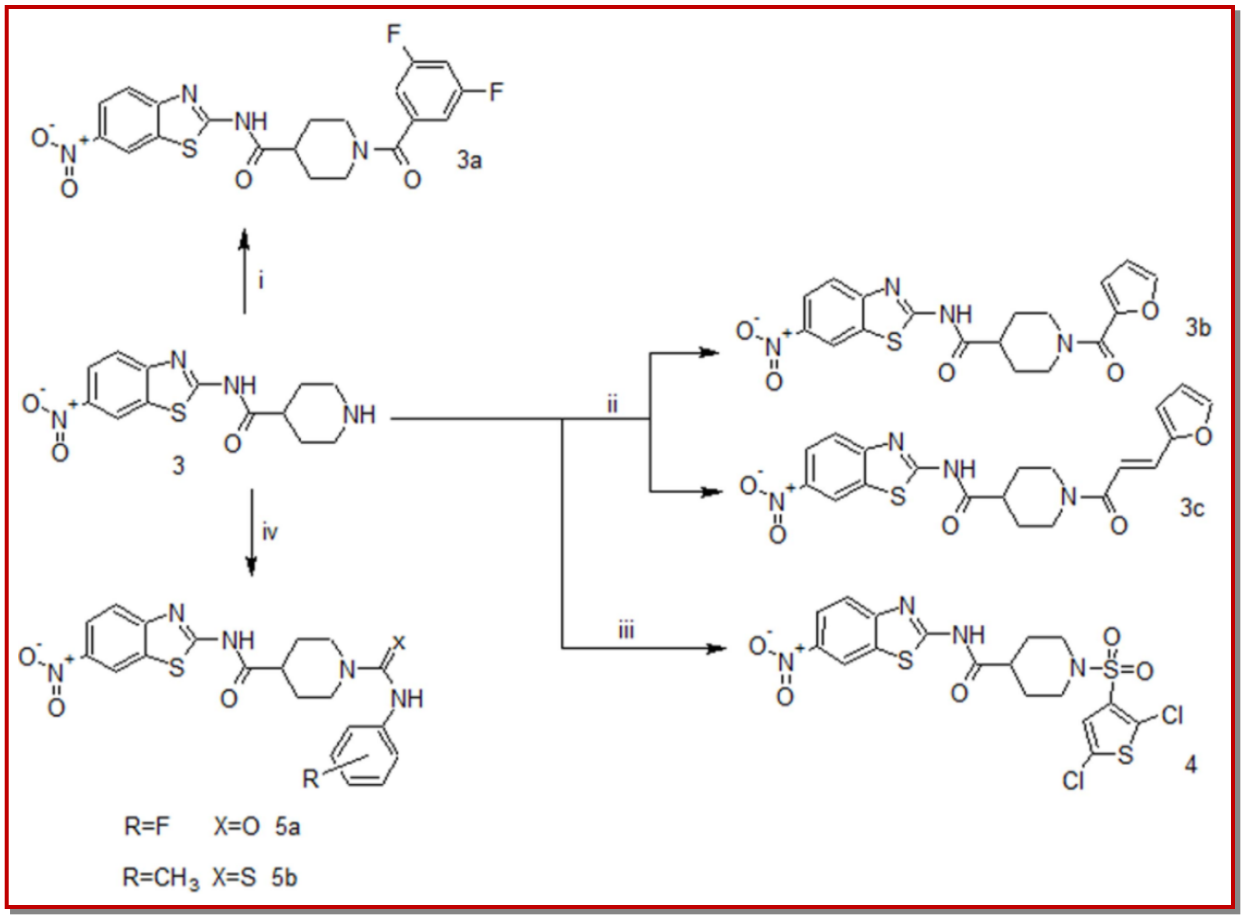
The synthetic routes for the preparation of intermediates and target compounds are shown in Scheme 1-2. The amide bond between N-boc-piperidine-4-carboxylic acid and 2-amino-6-nitrobenzothiazole (1) was synthesized by using reagent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI). The FT-IR value about 3194 cm^{-1} (NH, amide), 1682 cm^{-1} (C=O), 1324, 1521 cm^{-1} (NO_2 stretch) and ^{1}H NMR value δ 12.8 (s, 1H, amide NH) confirmed the resulting amide bond . The saturated HCl in ether solution was used for prepare the intermediate 3. The synthetic methods for making of intermediate 3 are given in Scheme 1. The compound **3** was taken as a common scaffold for the synthesis of new series of benzothiazole derivatives. The amide derivatives **3a** was prepared by using the reagent 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI). The corresponding acid chlorides were used for making of amide derivatives **3b**, and **3c**. For all the amide derivatives, the NH proton was observed at δ 12-12.8 as broad singlet in ^{1}H NMR. The synthetic methods for preparation of amide derivatives are given in Scheme 2.

The compound 4 was prepared by using 2,5-dichlorothiophene-3-sulfonyl chloride and compound **3**. The reaction was carried out in the presence of pyridine at 90°C. The FT-IR stretching frequency 1326 cm^{-1}, 1164 cm^{-1} (S=O) confirmed that there is the formation of sulfonamide derivative. The corresponding isocyanates and isothiocyanates respectively were used for prepare the urea and thiourea derivatives **5a** and **5b**. The ^{13}C-NMR showed peaks δ at 174-176 (C=O) and 180-191 (C=S) respectively for urea and thiourea compounds. The synthetic methods for making of sulfonamide, urea and thiourea derivatives are given in Scheme 2. All the above derivatives were characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR and LC-MS.

Anti-plasmodial activity of all synthesized compounds



Scheme 1: Reagents and conditions: (i) N-boc-piperidine-4-carboxylic acid, THF, EDCI, HOBt, TEA, O°C, 20 hours, N_2 atm; (ii) HCl in ether, 1,4-dioxane, O°C



Scheme 2: Reagents and conditions: (i) THF, EDCI, HOBt, TEA, O°C N_2 atm; (ii) TEA, THF, O°C, 20 hours; (iii) Dichloroethane, pyridine, 90°C; (iv) MDC, 0, N_2 atm

tested at concentration ranging from 3.15 µg/mL to 100 µg/mL and chloroquine used as a positive control. The IC_{50} values of benzothiazole derivatives against *P. falciparum* strains at 24 and 48 hours of parasitemia suppression are listed in Table I and II. It is cleared that the compound **3b** showed equal activity to positive control at 48 hours than 24 hours. The microscopic observation of anti-plasmodial treatment with benzothiazole derivatives against *P. falciparum* was presented in Figure 1.

Discussion

The present work demonstrated the anti-plasmodial activity of benzothiazole derivative tested on *Plasmodium falciparum*. The compound **3b** with furoyl group substitution was most effective against *P. falcipaurm* with IC_{50} value 24.4 µM (24 hours) and 12.3 µM (48 hours). The compound **4** with thiophene sulfonamide group substitution, was also effective against P. falcipaurm with IC_{50} value 45.2 µM (24 hours) and 19.4

Table I

% Suppresion of parasitemia at 24 hours

Samples	Concentration (µg/mL)						IC_{50} (µM)
	3.125	6.25	12.5	25	50	100	
3a	7.6 ± 0.1	15.6 ± 0.1	27.2 ± 0.1	32.0 ± 0.0	46.0 ± 0.0	58.2 ± 0.1	72.8
3b	36.0 ± 0.0	40.8 ± 0.1	47.9 ± 0.0	53.3 ± 0.1	65.0 ± 0.0	77.5 ± 0.1	24.4
3c	-	10.8 ± 0.1	16.9 ± 0.1	22.5 ± 0.2	28.9 ± 0.0	36.0 ± 0.0	>100
4	26.0 ± 0.2	31.2 ± 0.1	39.3 ± 0.1	47.2 ± 0.1	56.8 ± 0.1	68.4 ± 0.2	45.2
5a	15.3 ± 0.1	28.0 ± 0.0	39.1 ± 0.1	42.4 ± 0.1	49.8 ± 0.1	63.6 ± 0.1	58.3
5b	12.6 ± 0.1	29.1 ± 0.1	39.9 ± 0.0	48.4 ± 0.1	53.1 ± 0.1	65.4 ± 0.1	52.5
Chloroquine	30.4 ± 0.2	42.2 ± 0.0	49.8 ± 0.1	65.2 ± 0.1	72.9 ± 0.1	80.5 ± 0.2	17.6

Table II

% Suppresion of parasitemia after the treatment with benzothiazole derivatives at 48 hours

	Concentration (µg/mL)						IC_{50} (µM)
Samples	3.125	6.25	12.5	25	50	100	
3a	22.2 ± 0.2	30.0 ± 0.1	37.7 ± 0.1	48.0 ± 0.1	56.4 ± 0.2	66.4 ± 0.2	48.6
3b	38.6 ± 0.2	44.6 ± 0.1	52.1 ± 0.0	63.0 ± 0.0	70.4 ± 0.1	81.3 ± 0.1	12.3
3c	7.6 ± 0.1	12.0 ± 0.0	19.3 ± 0.1	26.7 ± 0.1	29.9 ± 0.0	36.9 ± 0.0	>100
4	36.6 ± 0.1	43.1 ± 0.0	49.8 ± 0.0	57.7 ± 0.1	69.2 ± 0.0	73.2 ± 0.1	19.4
5a	18.5 ± 0.1	29.3 ± 0.1	40.0 ± 0.0	48.9 ± 0.0	59.8 ± 0.1	70.2 ± 0.1	44.7
5b	21.8 ± 0.0	31.8 ± 0.1	38.9 ± 0.1	48.0 ± 0.0	66.3 ± 0.1	72.6 ± 0.2	39.2
Chloroquine	33.5 ± 0.1	44.7 ± 0.1	51.3 ± 0.1	67.0 ± 0.0	76.6 ± 0.1	83.2 ± 0.1	12.6

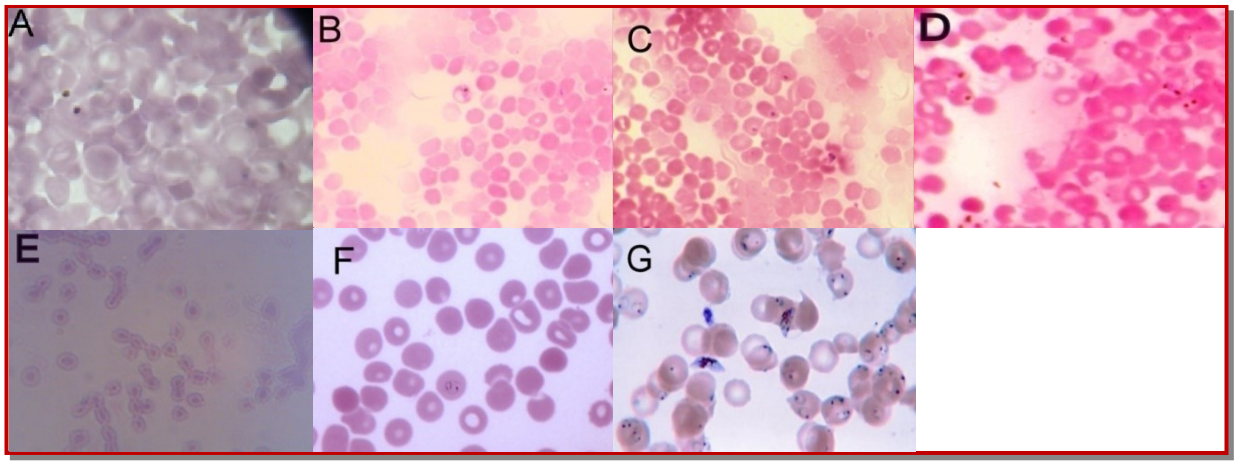


Figure 1: *In vitro* anti-plasmodial activity of benzothiazole derivatives. A. **3a**, B. **3b**, C. **4**, D. **5a**, E. **5b**, F. Chloroquine, G. Negative control

µM (48 hours). The compounds **3a**, **5a**, **5b** having amide, urea, and thiourea substituent showed moderate activity (IC_{50}<50) on the tested *P. faciparum*. The benzothiazole with furoyl acrylic acid substitution did not have marked activity since IC_{50} more than 100 µM .

Benzothiazole derivatives found to have diverse chemical reactivity and a broad spectrum of biological activity such as antitumor agents (Moustafa et al., 2014; Bradshaw et al., 1996), antimicrobial (Latrofa et al., 2005), analgesics, (Kaur et al., 2010), anti-inflammatory (Oketani et al., 2001), anti-HIV (Nagarajan et al., 2003). But benzothiazole have been poorly investigated their anti-plasmodial activity (Hout et al., 2004). We synthesized 2-substituted 6-nitrobenzothiazole with piperidine ring and tested on malaria parasites *P. falciparum*. The most interesting anti-parasite properties obtained with **3b** which inhibited *P. falciparum* growth at 24 and 48 hours. The compound **3b** showed chloroquine equal IC_{50} value at 48 hours. The second promising molecules, **4** showed good anti-plasmodial activity at 48 hours. The results observed in the present study confirmed that 2-substituted 6-nitrobenzothiazole had promising anti-malarial activity.

From the above results, it can be said that potency of suppression of parasitemia by **3b** and **4** may be due to presence of five member ring (furan, thiophene) which is attached with piperidine. Furthermore all moderate active benzothiazole compounds are have six member ring which is attached with piperidine ring. Thus ,the current work clearly indicates that the above benzothiazole derivatives **3b** and **4** could be a novel potent anti-malarial drug.

Conclusion

The present study describes the synthesis of 2-substituted-6-nitrobenzothiazole derivatives. The resulting compound was evaluated for their anti-plasmodial activity against plasmodium falciparum. The compound **3b** shown potent inhibition for the growth of parasitemia.

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Conflict of Interest

Authors declare no conflict of interest

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