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Research Article

New Benzoxazole Derivatives as Antiprotozoal Agents: *In Silico* Studies, Synthesis, and Biological Evaluation

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Background. Benzoxazole derivatives have different biological activities. In pursuit of designing novel chemical entities with antiprotozoal and antimicrobial activities, benzoxazolyl aniline was utilized as a privileged scaffold of a series of (3-benzoxazole-2-yl) phenylamine derivatives, 3-benzoxazoloyl acetamide, and butyramide derivatives. **Methods.** These novel analogs were synthesized in straightforward simple chemistry without any quantitative chromatographic separations in reasonable yields. The biological evaluation of all target compounds as potential antimalarial, antileishmanial, antitrypanosomal, and antimicrobial agents was performed by various well-established cell-based methods. **Results.** Compounds **6d** and **5a** showed promising biological screening data. The amidation of 3-benzoxazolyl aniline **1** with the chloroacetyl functional group resulted in a good antimalarial activity and showed moderate inhibitory activities against leishmanial and trypanosomal spp. Moreover, chloroacetyl functionalization of benzoxazolyl aniline serves as a good early goal for constructing and synthesizing new antimicrobial and antiprotozoal agents. The molecular docking study rationalizes the relative inhibitory activity of compound **5a** as an antimalarial agent with the deregulation of PfPNP activity which has emerged as a major mechanism of these targets.

1. Introduction

Malaria is a lethal illness initiated through the spreading of parasites via the nibbles of infected female mosquitoes. In 2015, the WHO reported that approximately half of the world's population (3.2 billion) was at threat of malaria with approximate deaths of 0.8–1.2 million per year [1]. The

African continent bears the brunt of this parasitic disease, with an estimated percentage of 90% of all malarial deaths being children under five years of age [2]. There is no licensed malaria vaccine available. Due to parasite resistance, the current effective drugs, such as artemisinin combination therapies (ACTs), are becoming less effective and sometimes lead to treatment failure [3] and relapse of malaria. Another

life-threatening neglected tropical disease, leishmaniasis, is triggered by several *Leishmania* parasite genus [4]. The two major types of leishmaniasis in humans are visceral, cutaneous leishmaniasis (CL), and leishmaniasis (VL) [5]. The later tropical disease is primarily triggered by *L. donovani* and can cause death in all situations if left unprocessed [6].

Alternatively, the growing incidence of infections with multidrug-resistant microbials has become essential problematic healthcare. Specifically, the development of multidrug-resistant pathogenic bacteria such as *Staphylococcus epidermidis*, vancomycin-resistant *Enterococcus*, and methicillin-resistant *Staphylococcus epidermis* is a significant challenge [7–9].

The oxazoles derivatives are considered the “isosteres of natural nucleotides” and became the target of many researchers to develop various synthetic analogs with substantial chemotherapeutic activities [10–14]. Many naturally and synthetically bioactive compounds containing benzoxazole scaffold registered to display a wide range of biological actions, for example, antimalarial [6], antileishmanial [6], antiviral [15, 16], and antimicrobial [17], along with inhibiting the activity of eukaryotic topoisomerase II enzyme [18–20] and activity against multidrug-resistance cancer cells [21–26].

A carboxylic polyether antibiotic calcimycin (Figure 1(a)) derived from *Streptomyces chartreuses* (NRRL 3882) is a natural benzoxazole derivative with very potent activity versus Gram-positive bacteria involving some *Micrococcus* and *Bacillus* [27, 28]. Also, *N*-(2-benzoxazole-2-ylphenyl)benzamide derivative (Figure 1(b)) is another natural antibiotic isolated from *Streptomyces* sp. (NRRL 12068) culture with significant antibacterial activity, remarkable activity against *Plasmodium falciparum* with a percent inhibition of 97 % (conc. 4.8 $\mu\text{g}\cdot\text{mL}^{-1}$), and promising antileishmanial activity (three-fold more potent than miltefosine which is an alkylphosphocholine agent with worthy activity against *Leishmania*) [5]. These facts have inspired the medicinal chemists to consider the benzoxazole nucleus as a privileged core structure for designing novel chemical entities with potential anti-infective, antiprotozoal, and antimicrobial activities.

In consideration of the aforementioned background and with the extension of our former investigations on benzoxazole moiety and its innate pharmacological activities [14, 29–33], we describe the preparation of a new series of compounds with 3-benzoxazolyl aniline scaffold (Figure 2). All the analogs were screened for their antimicrobial, antimalarial, antileishmanial, and antitrypanosomal activities.

2. Materials and Methods

2.1. Preparation of 2a-g. To a solution of 3-benzoxazol-2-ylphenylamine (**1**) (4.20 g, 0.02 mol) in mixture of ethanol/acetic acid (30 mL/0.5 mL), the aromatic aldehydic derivative (0.02 mol) was added. After four hours of reflux, the reaction mixture was cooled and the precipitate was filtrated out. 95% EtOH was used to recrystallize the precipitate to obtain imine analogs **2a-g** in pure forms and good yields. The ^1H NMR and ^{13}C DEPTQ-135 NMR screening were

carried out using CDCl_3 and $\text{DMSO}-d_6$ solvents and measured at δ scale. IR was performed using a KBr disc.

2.2. (3-Benzoxazol-2-yl-phenyl)-benzylidene-amine (2a). Solid white colored; yield (84%); mp 173–5 °C; IR: 3054 (CH aromatic) and 1609 (C=N) cm^{-1} ; ^1H NMR: 7.30–7.40 (m, 4H, benzoxazolyl H4,5,6,7), 7.52–7.60 (m, 3H, phenyl H3,4,5), 7.66–7.71 (m, 1H, phenylamine H5), 7.79–7.81 (m, H, phenylamine H4), 7.95–7.96 (m, 2H, phenylamine H2,6), 8.32 (d, $J=8.4$, 2H, phenyl H2,6), and 8.53 (s, 1H, N=CH); ^{13}C NMR: 110.25, 110.55, 114.67, 119.32, 119.91, 121.48, 124.21, 124.60, 125.02, 128.77, 128.91, 129.02, 129.42, 129.77, 131.40, 134.50, 161.42, and 192.44; calculated analysis of ($\text{C}_{20}\text{H}_{14}\text{N}_2\text{O}$): C, 80.52; H, 4.73; N, 9.39; present: C, 80.50; H, 4.70; N, 9.30.

2.3. (3-Benzoxazol-2-yl-phenyl)-(4-methyl-benzylidene)amine (2b). Solid white colored; yield: 82%; mp 183–5 °C; IR: 3053 (CH arom.), 2913 (CH ali.), and 1595 (N=C) cm^{-1} ; ^1H NMR: 2.49 (s, 3H, CH_3), 7.31–7.41 (m, 6H, benzoxazolyl H4,5,6,7 and phenyl H4,5), 7.61–7.79 (m, 2H, phenyl H2,6), 7.85 (d, 2H, $J=8.8$ Hz, tolyl H3,5), 8.31 (d, 2H, $J=8.8$ Hz, tolyl H2,6), and 8.49 (s, H, N=CH); ^{13}C NMR: 21.79, 21.91, 110.23, 110.54, 114.47, 116.90, 119.31, 119.89, 121.51, 124.22, 124.28, 124.58, 124.99, 128.76, 129.15, 129.42, 129.66, 129.73, 129.88, 133.25, 142.35, 145.58, 150.54, 161.35, and 163.70; calculated analysis of ($\text{C}_{21}\text{H}_{16}\text{N}_2\text{O}$): C, 80.75; H, 5.16; N, 8.97; present: C, 80.70; H, 5.20; N, 9.20.

2.4. (3-Benzoxazol-2-yl-phenyl)-(4-fluoro-benzylidene)amine (2c). Yellow solid colored, yield: 79%; mp 189–91 °C; IR: 3058 (CH arom.) and 1586 (C=N) cm^{-1} ; ^1H NMR: 7.19–7.40 (m, 6H, benzoxazolyl H4,5,6,7 and fluorophenyl H3,5), 7.60–7.62 (m, H, phenyl H5), 7.79–7.81 (m, 1H, phenyl H4), 7.91–7.98 (m, 2H, phenyl H2,6), 8.32 (d, 2H, fluorophenyl H2,6), and 8.48 (s, 1H, N=CH); ^{13}C NMR: 110.22, 114.67, 116.22, 116.50, 119.33, 119.92, 121.46, 124.20, 124.26, 124.62, 125.04, 128.79, 131.06, 131.15, 132.31, 142.22, 150.78, 154.65, 159.81, 163.73, and 166.25; calculated analysis of ($\text{C}_{20}\text{H}_{13}\text{FN}_2\text{O}$): C, 75.94; H, 4.14; N, 8.86; present: C, 75.70; H, 4.20; N, 8.90.

2.5. (3-Benzoxazol-2-yl-phenyl)-(4-methoxy-benzylidene)amine (2d). White solid colored; yield: 75%; mp 266–8 °C; IR: 3047 (CH arom.), 2928 (CH aliph.), and 1590 (N=C) cm^{-1} ; ^1H NMR: 3.91 (s, 3H, OCH_3), 7.02–7.06 (m, 2H, phenyl H4,5), 7.33–7.38 (m, 4H, benzoxazolyl H4,5,6,7), 7.60–7.61 (m, 1H, phenyl H6), 7.78–7.79 (m, 1H, phenyl H2), 8.08 (d, $J=8.8$ Hz, 2H, methoxyphenyl H3,5), 8.30 (d, $J=8.8$ Hz, 2H, methoxyphenyl H2,6), and 8.45 (s, 1H, N=CH); ^{13}C NMR: 55.61, 110.22, 114.32, 116.93, 119.32, 119.86, 121.51, 124.20, 124.95, 128.75, 129.40, 130.88, 132.02, 142.41, 149.65, 150.77, 160.64, 163.78, and 164.62; calculated analysis of ($\text{C}_{21}\text{H}_{16}\text{N}_2\text{O}_2$): C, 76.81; H, 4.91; N, 8.53; present: C, 76.70; H, 4.90; N, 8.60.

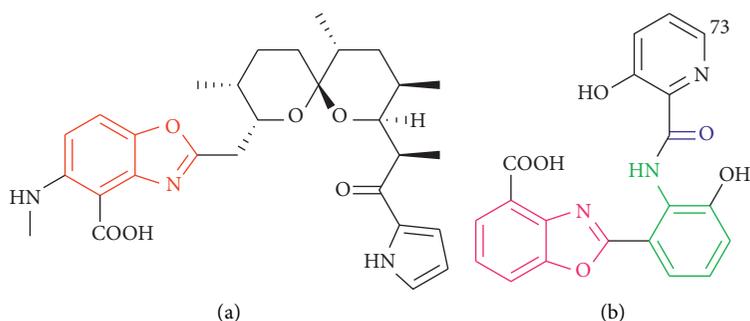


FIGURE 1: Structures of natural antibiotics containing benzoxazole nucleus.

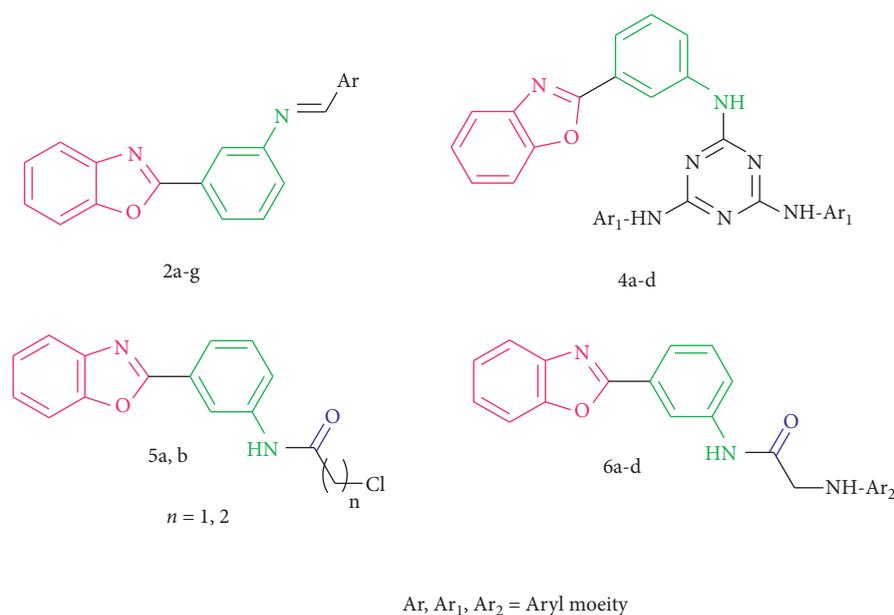


FIGURE 2: Design of target compounds containing 3-benzoxazol-2-ylphenyl moiety.

2.6. (3-Benzoxazol-2-yl-phenyl)-(4-chlorobenzylidene)-amine (2e). Pale yellow crystals; yield: 87%; mp 234–6°C; IR: 3054 (CH arom.) and 1612 (C=N) cm⁻¹; ¹HNMR: 7.27–7.31 (m, 4H, benzoxazolyl H4,5,6,7), 7.34–7.39 (m, 2H, phenyl H4,5), 7.59–7.62 (m, 1H, phenyl H6), 7.79–7.86 (m, 1H, phenyl H2), 7.95 (d, *J* = 8.4, 2H, chlorophenyl H2,6), and 8.49 (s, 1H, N=CH); ¹³CNMR: 110.23, 110.56, 114.67, 119.93, 121.47, 124.21, 124.28, 124.63, 125.07, 128.8, 129.23, 129.41, 129.48, 130.21, 130.93, 138.00, 150.0, and 159.87; EIMS (*m/z*): 332 (M⁺, 100%); calculated analysis of (C₂₀H₁₃ClN₂O): C, 72.18; H, 3.94; N, 8.42; present: C, 72.10; H, 4.10; N, 8.40.

2.7. (3-Benzoxazol-2-yl-phenyl) (3,4-difluorobenzylidene)-amine (2f). Yellow crystals; yield: 81%; mp 209–11 °C; IR: 3058 (CH arom.) and 1593 (C=C) cm⁻¹; ¹HNMR: 7.28–7.40 (m, 5H, benzoxazolyl H4,5,6,7 and fluorophenyl H5), 7.58–7.65 (m, 2H, phenyl H4,5), 7.79–7.81 (m, 1H, phenyl H6), 7.84–7.89 (m, 1H, phenyl H2), 8.32 (d, *J* = 8.4 Hz, 2H, fluorophenyl H2,6), and 8.45 (s, 1H, N=CH); ¹³CNMR: 110.22, 110.57, 114.67, 117.03, 117.85, 119.33, 119.95, 121.46,

124.20, 124.65, 124.99, 125.11, 126.04, 126.81, 128.81, 129.40, 142.19, 150.79, 151.50, 154.04, 158.61, and 162.77; calculated analysis of (C₂₀H₁₂F₂N₂O): C, 71.85; H, 3.62; N, 8.38; present: C, 72.70; H, 3.70; N, 8.50.

2.8. (3-Benzoxazol-2-yl-phenyl) (4-trifluoromethyl-benzylidene)-amine (2g). White crystals; yield: 83%; mp 240–2°C; IR: 3057 (CH arom.) and 1613 (C=N) cm⁻¹; ¹HNMR: 7.35–7.38 (m, 4H, benzoxazolyl H4,5,6,7), 7.59–7.61 (m, H, phenyl H-5), 7.71–7.81 (m, 3H, phenyl H2,4,6), 8.06 (d, 2H, *J* = 8 Hz, trifluoromethylphenyl H3,5), 8.32 (d, 2H, *J*₁ = 8 Hz, fluoromethylphenyl H2,6), and 8.55 (s, 1H, N=CH); ¹³CNMR: 110.58, 114.65, 119.32, 119.97, 121.49, 122.47, 124.20, 124.66, 125.14, 125.82, 126.11, 126.14, 128.81, 129.21, 129.93, 132.98, 133.31, 138.87, 142.19, 150.79, 154.08, 159.60, and 162.73; calculated analysis of (C₂₁H₁₃F₃N₂O): C, 68.85; H, 3.58; N, 7.65; present: C, 68.80; H, 3.60; N, 7.40.

2.9. Synthesis of (3-Benzoxazol-2-yl-phenyl) (4,6-dichloro [1,3,5]triazin-2-yl)amine (3). The solution of 3-benzoxazol-2-yl-phenylamine (1) (4.2 g, 0.02 mol; acetone, 20 mL) was

added dropwise to cyanuric chloride solution (3.68 g, 0.02 mol; acetone, 20 mL) and stirred at room temperature. After one hour, the mixture was evaporated under vacuum, washed, and crystallized from EtOH (70%) to obtain triazine 3. White solid; yield: 76%; mp 302–4 °C; IR: 3434 (NH), 3069 (CH arom.), and 1618 (N=C) cm^{-1} ; ^1H NMR: 7.40–7.43 (m, 2H, benzoxazolyl H5,6), 7.72–7.78 (m, 2H, benzoxazolyl H4,7), 7.85–7.87 (m, 2H, phenyl H2,6), 8.12–8.28 (m, 2H, phenyl, H4, 5), and 11.51 (s, 1H, NH); ^{13}C NMR: 111.31, 120.04, 121.41, 121.99, 125.36, 125.83, 128.64, 129.38, 141.24, 141.95, 150.40, 150.58, 154.49, 155.0, and 162.51; calculated analysis of ($\text{C}_{16}\text{H}_9\text{Cl}_2\text{N}_5\text{O}$): C, 53.65; H, 2.53; N, 19.55. Present: C, 53.60; H, 2.60; N, 19.60.

2.10. The General Method for Preparation of Compounds 4a–d. Triazine (3) (3.58 g, 0.01 mol) was mixed with K_2CO_3 (2.76 g) and appropriate aniline (0.023 mol) in anhydrous dioxane (25 mL). After heating under reflux for 12 h, the mixture was emptied into ice water, and the separated precipitate was filtrated and crystallized from EtOH to obtain triazine derivatives **4a–d**.

2.11. (3-Benzoxazol-2-yl-phenyl)-(4,6-diphenylamino- [1,3,5] triazin-2-yl)amine (4a). Solid buff colored; yield: 81%; mp 195–7 °C; IR: 3404 (NH), 3274 (NH), 3054 (CH arom.), and 1615 (N=C) cm^{-1} ; ^1H NMR: 7.14–7.16 (m, 4H, 2 phenyl-amino H2,6), 7.17–7.20 (m, 2H, two-phenylamino H4), 7.26–7.40 (m, 6H, two-phenylamino H3,5 and benzoxazolyl H5,6), 7.60–7.65 (m, 4H, phenyl H6 and benzoxazolyl H4,7, NH), 7.89–7.94 (m, 3H, phenyl, H2,4,5), and 8.19–8.24 (m, 2H, 2NH); ^{13}C NMR: 111.20, 119.89, 119.93, 120.30, 120.48, 121.11, 121.48, 122.81, 123.14, 125.48, 128.29, 128.95, 140.20, 142.25, 144.20, 150.63, 163.00, 164.48, and 164.61; calculated analysis of ($\text{C}_{28}\text{H}_{21}\text{N}_7\text{O}$): C, 71.32; H, 4.49; N, 20.79. Present: C, 71.40; H, 4.60; N, 20.60.

2.12. (3-Benzoxazol-2-yl-phenyl) (4,6-di(4-methylphenylamino)- [1,3,5]triazin-2-yl)amine (4b). Solid white colored; yield: 79%; mp 267–9 °C; IR: 3402, 3278 (2NH), 3028 (CH arom.), and 1614 (N=C) cm^{-1} ; ^1H NMR: 2.39 (s, 6H, CH_3), 7.03 (s, 2H, NH), 7.19 (d, 4H, $J=8.4$ Hz, two-tolyl H2,6), 7.28 (s, 1H, NH), 7.35–7.39 (m, 2H, benzoxazolyl H5,6), 7.48 (d, 4H, $J=8.4$ Hz, two-tolyl H3,5), 7.60–7.61 (m, 1H, phenyl H6), 7.77–7.79 (m, 3H, benzoxazolyl H4,7 and phenyl H2), and 8.20–8.22 (m, 2H, phenyl, H4,5); ^{13}C NMR: 20.98, 110.45, 119.64, 119.72, 124.49, 124.75, 128.56, 129.41, 133.42, 135.64, 142.02, 142.30, 144.00, 147.00, 149.00, 151.00, 152.00, 155.00, 157.00, 160.00, and 164.58; calculated analysis of ($\text{C}_{30}\text{H}_{25}\text{N}_7\text{O}$): C, 72.13; H, 5.04; N, 19.63; present: C, 72.30; H, 5.00; N, 19.60.

2.13. (3-Benzoxazol-2-yl-phenyl) (4,6-di(4-methoxyphenylamino) [1,3,5]triazin-2-yl)amine (4c). Solid Buff colored; yield: 69%; mp 248–50 °C; IR: 3401, 3284 (2NH), 2998 (CH arom.), and 1614 (C=N) cm^{-1} ; ^1H NMR: 3.34 (s, 6H, two- OCH_3), 6.92 (d, 4H, $J=8.8$, two-methoxyphenyl H2,6), 7.37–7.41 (m, 4H, benzoxazolyl H4,5,6,7), 7.65–7.84 (m, 6H,

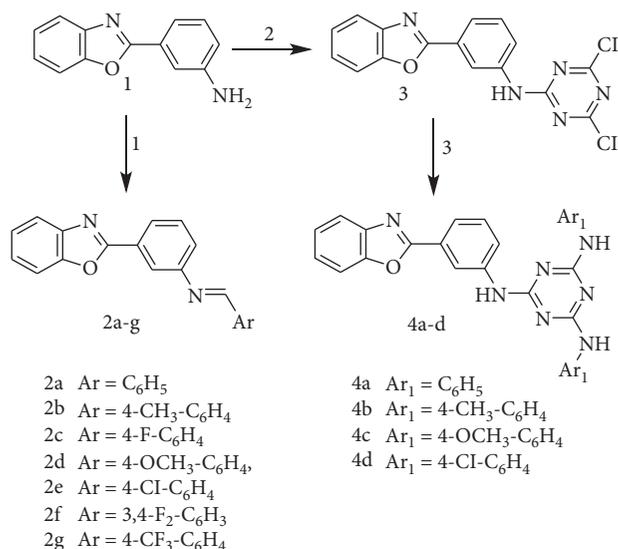
phenyl H2,6 and two-methoxyphenyl H3,5), 8.05–8.18 (m, 2H, phenyl H4,5), 9.10 (s, 2H, NH), and 9.55 (s, 1H, NH); ^{13}C NMR: 55.56, 111.13, 111.26, 114.06, 119.83, 119.83, 120.05, 125.15, 125.28, 125.36, 125.71, 128.85, 128.85, 133.57, 142.25, 144.25, 150.56, 163.09, 164.26, and 125.68; calculated analysis of ($\text{C}_{30}\text{H}_{25}\text{N}_7\text{O}_3$): C, 67.78; H, 4.74; N, 18.44; present: C, 67.80; H, 4.90; N, 18.40.

2.14. (3-Benzoxazol-2-yl-phenyl) (4,6-di(4-chlorophenylamino) [1,3,5]triazin-2-yl)amine (4d). Solid buff colored; yield: 72%; mp 265–7 °C; IR: 3414, 3201 (2NH), 3089 (CH arom.), and 1609 (C=N) cm^{-1} ; ^1H NMR: 7.40–7.41 (m, 4H, benzoxazolyl H4,5,6,7), 7.77–7.84 (m, 7H, phenyl H2,4,6 and two-chlorophenyl H2,6), 8.16–8.18 (m, 5H, two-chlorophenyl H3,5 and phenyl H5), 8.38 (s, 2H, NH), and 10.59 (s, 1H, NH); ^{13}C NMR: 111.25, 117.70, 119.60, 119.84, 120.04, 121.60, 121.75, 125.26, 125.69, 128.83, 129.22, 141.87, 142.07, 146.00, 150.59, 156.00, 160.59, 162.56, and 163.01; calculated analysis of ($\text{C}_{28}\text{H}_{19}\text{Cl}_2\text{N}_7\text{O}$): C, 62.23; H, 3.54; N, 18.14; present: C, 62.20; H, 3.60; N, 18.20.

2.15. The General Method of Chloroketones 5a&b Synthesis. To a stirred ice-cold solution of **1** (2.10 g, 0.01 mol) in anhydrous dimethylformamide (10 mL), the appropriate chloroacyl chloride (1.12 g) was added slowly, and then, adding K_2CO_3 (1.38 g) was added. The mixture was further stirred for 24 h at room temperature. At the end of the reaction, the mixture was emptied in ice water; the precipitate was filtrated, left to dry, and crystallized from CH_3OH to produce chloroketones **5a&b**.

2.16. N-(3-benzoxazol-2-yl-phenyl)-2-chloroacetamide (5a). Solid white colored; yield: 80%; mp 215–7 °C; IR: 3439(NH), 3098 (CH arom.), 2946 (CH Ali.), and 1673 (C=O), 1613 (N=C) cm^{-1} ; ^1H NMR: 4.33 (s, 2H, CH_2Cl), 7.37–7.42 (m, 2H, benzoxazolyl H5,6), 7.75–7.85 (m, 4H, benzoxazolyl H4,7 and phenyl H4,5), 8.17–8.19 (m, 2H, phenyl H2,6), and 10.69 (s, 1H, NH); ^{13}C NMR: 44.08, 111.25, 119.96, 120.06, 121.93, 125.27, 125.71, 128.79, 142.08, 142.19, 149.00, 135.00, 150.61, 162.54, and 165.63; EIMS (m/z): 286 (M^+ , 100%); calculated analysis of ($\text{C}_{15}\text{H}_{11}\text{ClN}_2\text{O}_2$): C, 62.84; H, 3.87; N, 9.77. Present: C, 62.70; H, 3.60; N, 10.10.

2.17. N-(3-benzoxazol-2-yl-phenyl)-4-chlorobutyramide (5b). Solid greyish green colored; yield: 77%; mp 212–4 °C; IR: 3465(NH), 3120 (CH arom.), 2958, 2917 (CH Ali.), and 1665 (C=O), 1599 (C=N) cm^{-1} ; ^1H NMR: 2.03–2.09 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{Cl}$), 2.55 (t, 2H, $J=7.2$, $\text{CH}_2\text{CH}_2\text{CH}_2\text{Cl}$), 3.72 (t, 2H, $J=6.4$, $\text{CH}_2\text{CH}_2\text{CH}_2\text{Cl}$), 7.38–7.42 (m, 2H, benzoxazolyl H5,6), 7.74–7.78 (m, 2H, benzoxazolyl H4,7), 7.81–7.85 (m, 2H, phenyl H4,5), 8.13–8.15 (m, 2H, phenyl H2,6), and 10.36 (s, 1H, NH); ^{13}C NMR: 28.21, 33.96, 45.44, 110.80, 111.19, 115.32, 119.31, 119.60, 119.97, 121.13, 124.71, 124.89, 125.21, 125.59, 128.67, 129.40, 142.09, 142.93, 150.56, 162.69, and 171.27; EIMS (m/z): 314 (M^+ , 33%) and 316 (M^++2 , 12.54%); calculated analysis of ($\text{C}_{17}\text{H}_{15}\text{ClN}_2\text{O}_2$): C, 64.87; H, 4.80; N, 8.90. Present: C, 74.80; H, 4.90; N, 8.70.

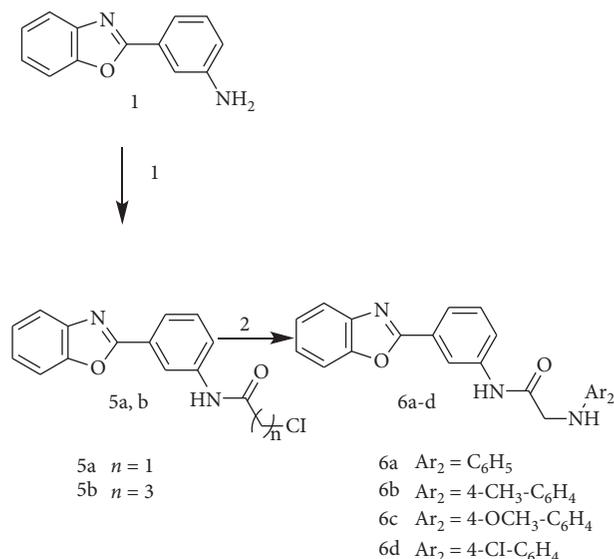


SCHEME 1: Synthetic pathway of **2a-g** and **4a-d**. **Conditions and reagents:** (1) ArCHO, CH₃CH₂ OH, CH₃COOH, reflux, 4h; (2) cyanuric chloride, acetone, rt, 1h; and (3) aryl amine, K₂CO₃, dioxane, reflux, 12h.

2.18. *General Synthetic Method of Compounds 6a-d*. A mixture of the appropriate amines (0.03 mol), **5a** (5.72 g, 0.02 mol), EtOH (40 mL), and a few drops of triethylamine was heated for 12 h under reflux. The solution was cooled, and the precipitate was filtrated, left to dry, and recrystallized of EtOH to give **6a-d**.

2.19. *N*-(3-benzoxazol-2-yl-phenyl)-2-phenylamino-acetamide (**6a**). Grey solid; yield: 87%; mp 234–7 °C; IR: 3383, 3314 (NH), 3097 (CH arom.), 2929 (CH Ali.), 1675 (C=O), and 1606 (C=N) cm⁻¹; ¹HNMR: 3.99 (s, 2H, CH₂), 4.36 (s, 1H, NH), 6.74 (d, 2H, *J* = 8 Hz phenylamino H_{2,6}), 6.90–6.93 (m, 1H, phenylamino H₄), 7.28–7.45 (m, 6H, benzoxazolyl H_{4,5,6,7} and phenylamino H_{3,5}), 7.74–7.82 (m, 2H, phenyl H-4,5), 8.22–8.30 (m, 2H, phenyl H-2,6), and 8.83 (s, 1H, NH); ¹³CNMR: 50.13, 110.53, 113.70, 119.66, 119.85, 120.13, 122.50, 123.02, 124.58, 124.98, 128.65, 129.70, 134.25, 140.19, 141.50, 144.00, 146.81, and 169.16; calculated analysis of (C₂₁H₁₇N₃O₂): C, 73.45; H, 4.99; N, 12.24. Present: C, 73.50; H, 4.90; N, 12.20.

2.20. *N*-(3-benzoxazol-2-yl-phenyl)-2-(4-methylphenylamino)acetamide (**6b**). Greyish-white solid; yield 80%; mp 230–2 °C; IR: 3381, 3311 (2NH), 3026 (CH arom.), 2917 (CH Ali.), and 1675 (C=O) cm⁻¹; ¹HNMR: 2.29 (s, 3H, CH₃), 3.94 (s, 2H, CH₂), 4.30 (s, 1H, NH), 6.65 (d, 2H, *J* = 8 Hz, 4-methylphenylamino H_{2,6}), 7.08 (d, 2H, *J* = 8 Hz, 4-methylphenylamino H_{3,5}), 7.25–7.58 (m, 4H, benzoxazolyl H_{4,5,6,7}), 7.58 (s, 1H, phenyl H₂), 7.74–7.82 (m, 2H, phenyl H_{4,5}), 8.18–8.28 (m, 2H, phenyl H_{2,6}), and 8.94 (s, 1H, NH); ¹³CNMR: 20.93, 50.00, 110.56, 119.34, 119.59, 119.87, 119.99, 121.92, 123.06, 124.61, 125.02, 128.70, 128.80, 129.63, 130.23, 130.30, 139.55, 140.08, 151.79, and 163.00; calculated



SCHEME 2: Synthetic pathway of **5a-b** and **6a-d**. **Condition and reagents:** (1) acid chloride, K₂CO₃, dimethyl formamide, rt, 24h; (2) aryl amines, TEA, CH₃CH₂ OH, reflux, 12h.

analysis of (C₂₂H₁₉N₃O₂): C, 73.93; H, 5.36; N, 11.76; present: C, 73.80; H, 5.30; N, 11.90.

2.21. *N*-(3-benzoxazol-2-yl-phenyl)-2-(4-methoxyphenylamino)acetamide (**6c**). Greyish-white solid; yield 79%; mp 209–12 °C; IR: 3427, 3380 (2NH), 3000 (CH arom.), 2924 (CH Ali.), 1675 (C=O), and 1613 (C=N) cm⁻¹; ¹HNMR: 3.63 (s, 3H, OCH₃), 3.87 (s, 2H, CH₂), 5.675–5.68 (s, 1H, NH), 6.58 (d, 2H, *J* = 8.8 Hz, 4-methoxyphenylamino H_{2,6}), 6.75 (d, 2H, *J* = 8.8 Hz, 4-methoxyphenylamino H_{3,5}), 7.39–7.42 (m, 2H, benzoxazolyl H_{5,6}), 7.75–7.88 (m, 4H, benzoxazolyl H_{4,7} and phenyl H_{4,5}), 8.14–8.16 (m, 2H, phenyl H_{2,6}), and 10.31 (s, 1H, NH); ¹³CNMR: 48.80, 55.76, 111.23, 113.89, 115.08, 119.83, 120.01, 121.39, 125.66, 125.66, 128.70, 142.10, 142.57, 142.84, 150.59, 151.73, 162.66, and 170.79; calculated analysis of (C₂₂ H₁₉N₃O₃): C, 70.76; H, 5.13; N, 11.25; present: C, 70.70; H, 5.10; N, 11.10.

2.22. *N*-(3-benzoxazol-2-yl-phenyl)-2-(4-chlorophenylamino)acetamide (**6d**). White solid; yield 85%; mp 195–7 °C; IR: 3396, 3267 (2NH), 3060 (CH arom.), 2930 (CH Ali.), 1670 (C=O), and 1607 (C=N) cm⁻¹; ¹HNMR: 3.94 (s, 2H, CH₂), 6.29 (s, 1H, NH), 6.62 (d, 2H, *J* = 8.4 Hz, 4-chlorophenylamino H_{2,6}), 7.13 (d, 2H, *J* = 8.4 Hz, 4-chlorophenylamino H_{3,5}), 7.39–7.41 (m, 2H, benzoxazolyl H_{5,6}), 7.74–7.85 (m, 4H, benzoxazolyl H_{4,7} and phenyl H_{4,5}), 8.14–8.16 (m, 2H, phenyl H_{2,6}), and 10.43 (s, 1H, NH); ¹³CNMR: 47.61, 111.25, 114.16, 119.87, 119.99, 121.43, 125.32, 125.73, 128.73, 129.07, 142.01, 142.51, 147.68, 150.56, 152.00, 157.00, 162.64, 167.00, and 170.15; EIMS (*m/z*): 377 (M⁺, 7.29%) and 379 (M⁺+2, 1.77%); calculated analysis of (C₂₁H₁₆ClN₃O₂): C, 66.76; H, 4.27; N, 11.12; present: C, 67.60; H, 4.10; N, 11.20.

2.23. In Vitro Antimicrobial Activity. All fungal and bacterial strains have been obtained from the ATCC (American Type Culture Collection). A total of five fungal strains were selected for testing their vulnerability to the target final compounds. These strains comprised *C. krusei* (ATCC 6258), *C. albicans* (ATCC 90028), *C. glabrata* (ATCC 90030), *Cryptococcus neoformans* (ATCC 90113), and *Aspergillus fumigatus* (ATCC 204305). We also assessed the sensitivity of five bacterial strains to the final target compounds. These strains comprised methicillin-resistant *S. aureus* (ATCC 33591) (MRSA), *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 35218), *M. intracellulare* (ATCC 23068), and *S. aureus* (ATCC 29213), and vulnerability testing was carried out using the Clinical and Laboratory Standards Institute-(CLSI-) adapted procedure [34]. A modified Franzblau method was applied to test the target compounds reactivity against *Mycobacterium intracellulare* [35]. All tested compounds were diluted serially utilizing NaCl (0.09%, solution) containing DMSO (20%, dimethyl sulfoxide) and transmitted in duplicate to microplates (96-well). Optical density was applied to correct the microbial inoculum at 630 nm of microbe suspension. In the evaluation protocols, two positive controls were used. Amphotericin B (ICN Biomedicals, Ohio) for fungi was used for susceptibility test of fungal strains, and ciprofloxacin (ICN Biomedicals, Solon, OH, USA) was used for the susceptibility test of bacterial strains. Either the 544ex/590E or BioTek Power Wave XS Plate Reader (Bio-Tek Instruments, Vermont) (*A. fumigatus*, *M. intracellulare*) was used to read all organisms before and after the incubation period, using the Polarstar Galaxy Plate Reader (BMG Lab Technologies, Germany). MBC (minimum bactericidal concentration) and MFC (Minimum fungicidal concentration) can be described as the lowermost concentration of the tested substance that kills the organism. The MFC and MBC have been established by taking 5 μ L of the individual well and transferred to agar. At 35 °C and after 48 h of incubation, the microdilution trays were investigated. From the dose-response curves, IC 50 (half-maximal inhibitory concentration) values were assessed to reduce cell viability by a percentage against test serial dilutions. Ciprofloxacin showed IC₅₀ values of 0.214, 0.06, 0.06, 0.10, and 0.01 μ M against *M. intracellulare*, MRSA, *E. coli*, *St. aureus*, and *P. aeruginosa*, respectively. Amphotericin B exhibited IC₅₀ values of 0.17, 0.57, 0.22, 0.17, and 1.30 μ M against *Cryptococcus neoformans*, *Aspergillus fumigatus*, *C. krusei*, *C. glabrata*, and *C. albicans*, correspondingly. A negative control dimethyl sulfoxide was selected.

2.24. In Vitro Screening of the Antimalarial Effect. The antimalarial activity was *in vitro* measured against two strains, *Plasmodium falciparum* (chloroquine resistant (W2) and chloroquine sensitive (D6)), supplied from the Division of Experimental Therapeutics, Walter Reed Army Institute of Research. The antimalarial activity assessment depends on the measurement of LDH (plasmodial lactate dehydrogenase) enzymatic action. A red blood cells (RBCs) suspension infected by both *Plasmodium falciparum* strains (W2 and D6) (with 2% parasitemia, at 200 μ l and two percentage of

hematocrit in RPMI 1640 medium supplemented with ten percentage of human serum and sixty μ g/ml amikacin) is added to the flat bottom microplates (96-well) containing 10 μ l of serially diluted test samples. At 37°C for 72 h, the plate is incubated in a modular incubation chamber flushed with a gas blend of ninety percentage of N₂, five percent of O₂, and five percent of CO₂. The Makler and Hinrichs procedure was followed to establish the plasmodial LDH action [36]. For half hour, a mixture of 100 μ l of Malstat reagent and twenty μ L of the incubation mixture were incubated at 25°C. 20 μ L of a 1:1 mixture of nitroblue tetrazolium (NBT) test, which measures ROS produced by spermatozoa, and phenazine ethosulfate, which is generally used to detect NO reductase activity in concert with ascorbic acid (Sigma), was added. In the dark, the plate was then incubated for an extra 60 minutes. The reaction was stopped by adding 100 μ l of five percent of CH₃COOH, and the plate was measured at 650 nm. The IC₅₀ data were collected via plotting the percentage of growth versus test concentrations. The positive controls in this assay protocol are artemisinin and chloroquine. However, DMSO is used for the placebo-controlled studies.

An *in vitro* cytotoxicity investigation against mammalian cells SI (selectivity index) determination of the test compounds was conducted. The screening was performed using Vero cells (monkey kidney fibroblasts from the American Type Culture Collection). At a density of twenty-five thousand cells per well, the mammalian cells were scattered and stored at 37°C for 24 h. Variable dilutions were added, and the plates were stored for 48 h. The neutral red assay was followed to determine the number of viable cells, and IC₅₀ data were then generated from dose-response curves [37].

2.25. In Vitro Assessment of Antileishmanial And Antitrypanosomal Activities. The *in vitro* antitrypanosomal and antileishmanial activities of target synthesized derivatives were investigated using stock 2 mg/mL concentration of the target compounds in DMSO. Four serial dilutions of all tested compounds were performed using an incomplete RPMI medium.

Three *Leishmania* species (*Leishmania donovani* Amastigote/THP1 cells, *Leishmania donovani* Amastigote, and *Leishmania donovani* Promastigote) were selected to investigate the antileishmanial activity, and one trypanosomal species (*Trypanosoma brucei*) was selected to investigate the antitrypanosomal activity using the Alamar Blue assay protocol [38–40].

In the exponential phase, *Leishmania donovani* promastigotes (three-day-old culture) were diluted with RPMI medium to 1 \times 10⁶ cells/ml for promastigote assays. A *Leishmania donovani* axenic amastigotes three-day-old culture was diluted with the same medium to 2 \times 10⁶ cells/ml for axenic amastigote assays. In the exponential phase, *Trypanosoma brucei* (two-day-old culture) was diluted with Iscove's Modified Dulbecco's Medium (IMDM), which is an adapted DME medium containing additional amino acids, sodium pyruvate, high glucose (4,500 mg/L), zwitterionic sulfonic acid buffering agent (4-(2-hydroxyethyl)-1-

piperazineethanesulfonic acid), selenium, and others, to 5×10^3 cells/ml for the antitrypanosomal assay.

The test compounds with three different serial dilutions were added to the *Trypanosoma brucei* trypomastigote, *Leishmania donovani* axenic amastigote, and *Leishmania donovani* promastigote cultures. All samples have been assessed at three serial dilutions starting from 10 $\mu\text{g}/\text{mL}$ to 0.4 $\mu\text{g}/\text{mL}$. At 26°C for 72 hours (37°C for *Trypanosoma brucei* trypomastigotes and axenic amastigotes), the plates were incubated, and by Alamar Blue assay as previously reported, the growth of the parasites in cultures was established [38–40].

Employing a newly developed parasite-rescue and transformation assay, samples were tested versus *Leishmania donovani* intracellular amastigotes in THP1 cells [41]. In the exponential phase, a four-day-old THP1 cell culture was diluted with a growth medium called the RPMI medium to 2.5×10^5 cells/ml.

To the final concentration of 25 ng/ml, PMA was added. In experimental culture plates, the culture treated with PMA was distributed and incubated overnight in a five percentage of CO₂ incubator at 37°C. A serum-free medium was then used to wash the plates by differentiated cells of THP1. For the THP1 toxicity assay, serial dilutions of target derivatives were added upon differentiated cells of THP1. For 48 hours at 37°C, the plates were anaerobically incubated using a five percentage of CO₂ incubator. Alamar Blue assessment was followed for determination of the cell growth, and the IC₉₀ data were calculated from the dose-response curves.

2.26. Molecular Modeling Study. The protein structure for PfPNP is available at the protein bank database, and the crystal structure (PDB ID: 3PHC) was downloaded from the protein databank repository [42]. PrepWizard (Protein Preparation Wizard) of the Schrödinger suite was used to prepare the protein structure. To do so, adding missing hydrogen atoms, relaxing the protein-ligand complex, assigning HBs, and adjusting bond orders [41] were carried out. Prime was applied to fill amino-acid side chains and the missing loops [43]. The cocrystallized ligand coordinates were used to assign the docking receptor grid center. Through scaling the VDW radius by a partial charge cutoff of 0.25 and factor of 1.0, the potential of the receptor nonpolar parts was softened. For optimum HB shape with docked poses predicted, rotation of the receptor SH and OH groups was permitted using the OPLS3 force field. LigPrep of Schrödinger was performed for ligand preparation [44, 45]. To produce potential ligand states at pH 7.4, Epik was used, and during ligand preparation, ligand stereogenic centers were maintained [46]. The glide SP (standard precision) mode was applied to execute virtual compounds docking; consequently, the best five poses were selected [47].

3. Results and Discussion

3.1. Chemistry. In Scheme 1, synthesis of oxazole derivatives **2a–g**, **3**, and **4a–d** is shown. Simple and straightforward Schiff bases **2a–g** were prepared by condensation of the 3-

benzoxazolyl aniline **1** with appropriate benzaldehyde under reflux condition in absolute ethanol and with 4–5 drops of glacial acetic acid. Another set of triazine derivatives **4a–d** was synthesized through successive nucleophilic substitution of cyanuric chloride with different aryl amines in one pot [48]. Cyanuric chloride was firstly stirred with compound **1** at room temperature for 1 hour in acetone to afford compound **3**, followed by heating with the suitable aromatic amine under reflux in dioxane to yield the triazine derivatives **4a–d**.

In Scheme 2, a straightforward reaction of compound **1** with acid chlorides (namely, chloroacetyl chloride and chlorobutyl chloride) at room temperature in DMF containing potassium carbonate afforded amides **5a,b** in quantitative yields is shown. Compound **5a** was further derivatized with aromatic amines via nucleophilic substitution of the chlorine atom in compound **5a** to yield compounds **6a–d** [33, 49–52]. The structures of all newly prepared derivatives were described and verified with different spectroscopic tools.

3.2. In Vitro Antimicrobial Activity. The antimicrobial effect was evaluated for all the synthesized benzoxazolyl aniline analogs. Antimicrobial actions were assessed against the fungal strains of *Cryptococcus neoformans*, *C. krusei*, *C. albicans*, *C. glabrata*, and *Aspergillus fumigatus* and the bacterial strains of *Mycobacterium intracellulare*, *Pseudomonas aeruginosa*, methicillin-resistant *S. aureus* (MRSA), *Staphylococcus aureus*, and *Escherichia coli*. Drug controls (amphotericin for fungi and ciprofloxacin for bacteria) was incorporated in each test as positive controls [35].

All compounds did not exhibit antimicrobial activities, whether for bacteria or fungi, up to 20 $\mu\text{g}/\text{mL}$ concentration, except 2-chloroacetamide **5a**, with an IC₅₀ value of 22.3 μM against *C. neoformans*.

3.3. Antiprotozoal Activity. All the novel prepared derivatives were biologically evaluated *in vitro* against resistant (W2) and chloroquine-sensitive (D6) strains of *Plasmodium falciparum* as potential antimalarial agents [35–37]. Three compounds (**4a**, **5a**, and **6d**) displayed promising anti-protozoal activity among the other target compounds, and their screening data with the two positive controls (chloroquine and artemisinin) are depicted clearly in Table 1.

Amongst the synthesized derivatives, N-(3-benzoxazol-2-yl-phenyl)-2-chloroacetamide (**5a**) exhibited the most prominent activity against both D6 and W2 strains with IC₅₀ values of 5.1 and 2.2 μM , respectively. Compound **6d** showed comparable antimalarial activities to compound **5a** against the both strains with IC₅₀ values of 5.5 and 4.1 μM , respectively.

All derivatives were biologically evaluated against two *Leishmania* spp. (*L. donovani* amastigote and *L. donovani* promastigote) and one *Trypanosoma* spp. (*T. brucei*) (Table 2) [38–40].

Compound **6d** showed moderate antileishmanial activity against both *L. donovani* amastigotes and promastigotes with IC₅₀ values of 17.9 and 23.7 μM , respectively, along with

TABLE 1: Antimalarial action of the potential derivatives.

Compound	<i>P. falciparum</i> , D6		<i>P. falciparum</i> , W2		VERO, IC ₅₀ (μM)
	IC ₅₀ (μM)	SI	IC ₅₀ (μM)	SI	
4a	9.1	>1.1	10	>1	>10.1
5a	5.1	1.2	2.2	2.8	6.3
6d	5.5	0.8	4.1	1.1	4.7
Chloroquine	0.03	—	0.31	—	NC
Artemisinin	0.02	—	0.01	—	NC

NC: not cytotoxic; W2, chloroquine-resistant strain; D6, chloroquine-susceptible strain; selectivity index (SI) (IC₅₀ for Vero cells/IC₅₀ for *P. falciparum*).

TABLE 2: Antileishmanial and antitrypanosomal activity of compounds 5-6.

Comp.	<i>L. donovani</i> promastigote		<i>L. donovani</i> amastigote		<i>L. donovani</i> Amastigote + THP		<i>T. brucei</i>		THP	
	^a IC ₅₀	^b IC ₉₀	IC ₅₀	IC ₉₀	IC ₅₀	IC ₉₀	IC ₅₀	IC ₉₀	IC ₅₀	IC ₉₀
5a	15.1	—	28.3	32.6	6.04	10.4	5.38	6.43	11.3	24.0
6a	—	—	—	—	—	—	16.8	24.1	5.02	—
6c	—	—	—	—	—	—	15.7	24.5	—	—
6d	23.7	—	17.9	25.6	20.6	—	2.75	3.81	—	—
Amb	0.178	0.229	0.333	0.390	0.159	0.230	NT	NT	—	—
Pm	4.88	7.62	29.2	—	3.08	17.3	NT	NT	—	—
DFMO	NT	NT	NT	NT	NT	NT	21.7	50.3	NT	NT

^aIC₅₀: concentration causing 50% growth inhibition; ^bIC₉₀: concentration causing 90% growth inhibition; Amb = amphotericin B; Pm = pentamidine; DFMO = difluoromethylornithine.

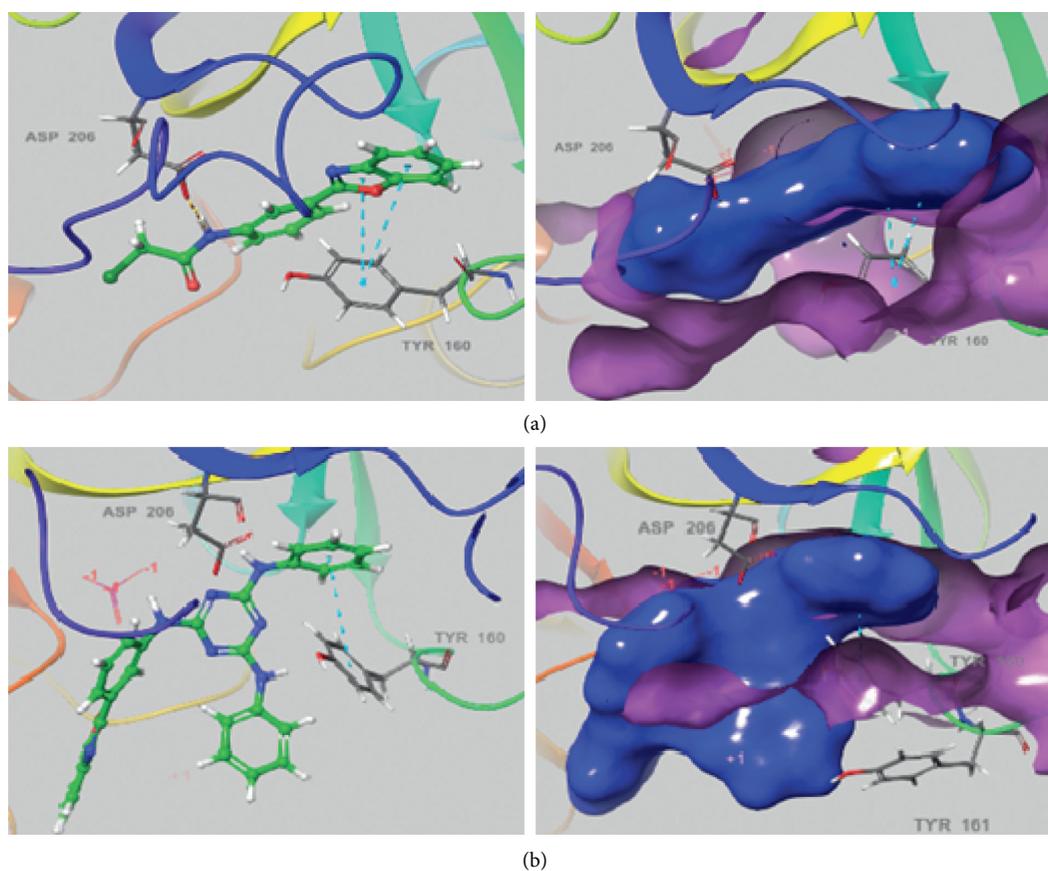


FIGURE 3: Binding modes of compounds 5a (a) and 4a (b) into the PfPNP active site; along with the interacting amino acids represented as labeled tube models, in a ball and stick model (left), the ligands are depicted. The binding cavities' channel surfaces are presented in purple, and the ligand surfaces are represented in blue (right).

potent antitrypanosomal activity with IC_{90} and IC_{50} values of 3.81 and 2.75 μM , respectively. Compound **5a** showed moderate antileishmanial effect against *L. donovani* promastigote and amastigotes with IC_{50} values of 15.1 and 28.3 μM , respectively, and antitrypanosomal activity against *T. brucei* with IC_{50} and IC_{90} values of 5.38 and 6.43 μM , respectively.

3.4. Docking Studies. One important target for antimalarials is PfPFP (P. falciparum purine nucleoside phosphorylase), the key enzyme in purine recycling and salvage. Inhibition of PfPFP results in parasite death through purine starvation, since *P. falciparum* is a purine auxotroph due to lack of the *de novo* purine biosynthetic pathway [41]. PfPFP catalyzes inosine phosphorolysis into ribose-1-phosphate and hypoxanthine, the primary purine substrate for the salvage pathway [53]. Therefore, we considered PfPFP as a molecular target of the proposed synthesized compounds. The crystal structure of PfPFP is available in the protein data bank (PDB) with PDB ID of 3PHC [54]. The crystal structure of PfPFP showed a homohexamer (in contrast to its human analog, which is a homotrimer [55]), and it is organized as a trimer of dimers, where in each monomer, the active site is completely occupied via the cocrystallized ligand (immucillin) and one sulfate [41]. Two analogous catalytic sites are localized between each of the dimer pairs and are correlated by a noncrystallographic two-fold axis. The virtual docking was performed for the most active compounds (**4a** and **5a**) in order to rationalize the activity of these compounds against malaria [56]. The docking simulations showed that compound **5a** was located properly at the binding site. Moreover, the molecular docking of the compounds **5a** and **4a** into the PfPFP active site showed superior binding affinity of **5a** than **4a**. The possible steric hindrance effect of 4,6-diphenylamino-triazine substitution of compound **4a** could negatively affect the binding affinity into the active site, which may explain the reduced antimalarial activity for this compound. The benzoxazole moiety was embedded into the hydrophobic pocket delimited by Pro209, Trp212 Tyr160, Met159, Val181, and Met183 and oriented to establish two π - π stacking interactions with Tyr160 and a hydrogen bond with Asp206 (Figure 3). Mutation studies verified that Asp206 is crucial for catalysis [56].

4. Conclusions

In summary, simplified novel analogs of naturally occurring antibiotic benzoxazole were prepared and assessed for their antimicrobial, antileishmanial, antimalarial, and antitrypanosomal activities. 3-Benzoxazolyl aniline amidation with chloroacetyl resulted in promising antifungal and antimalarial activities and showed moderate inhibitory activities against *Leishmania* and *Trypanosoma* spp. However, the substitution at the amino group with other substituents resulted in diminished inhibitory activities, except the compound **6d** that showed good antitrypanosomal activity. Our findings highlight the importance of the chloroacetyl functionalization of

benzoxazolyl aniline and provide a good starting point for designing potent and novel antimicrobial and anti-protozoal agents. Further work is being directed toward enhancing the activity and selectivity.

Data Availability

Data are uploaded as a supplementary file.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

The supplementary data contain NMR, IR, and mass spectroscopy charts of the new synthesized compounds. (*Supplementary Materials*)

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