Hindawi
Journal of Chemistry
Volume 2021, Article ID 6631868, 11 pages
https://doi.org/10.1155/2021/6631868

Research Article

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

Mohamed A. Abdelgawad,[1] **Mohammad M. Al-Sanea**,[1] **Mohamed A. Zaki**,[2,3,4] **Enas I. A. Mohamed**,[2] **Shabana I. Khan**,[4] **Babu L. Tekwani**,[4] **Amar G Chittiboyina**,[4] **Ikhlas A Khan**,[4,5] **Tarfah Al-Warhi**,[6] **Nada Aljaeed**,[6] **Ohoud J. Alotaibi**,[6] **Abdulsalam A. M. Alkhaldi**,[7] **and Heba A. H. Elshemy**[8]

[1]*Department of Pharmaceutical Chemistry, College of Pharmacy, Jouf University, Sakakah, Aljouf 72341, Saudi Arabia*
[2]*Department of Pharmacognosy, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt*
[3]*Research Institute of Medicinal and Aromatic Plants, Beni Suef University, Beni Suef 62514, Egypt*
[4]*National Centre for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, Oxford, Mississippi 38677, USA*
[5]*Division of Pharmacognosy, Department of Biomolecular Sciences, School of Pharmacy, University of Mississippi, MS 38677, USA*
[6]*Department of Chemistry, College of Science, Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia*
[7]*Biology Department, College of Science, Jouf University, Sakakah, Aljouf 72341, Saudi Arabia*
[8]*Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt*

Correspondence should be addressed to Mohamed A. Abdelgawad; mohamedabdelwahab976@yahoo.com

Received 26 November 2020; Revised 2 February 2021; Accepted 19 February 2021; Published 3 March 2021

Academic Editor: Teodorico C. Ramalho

Copyright © 2021 Mohamed A. Abdelgawad et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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 S*taphylococcus 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 $\mu g \cdot mL^{-1}$), 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-yl-phenylamine (**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 ^{1}HNMR and ^{13}C DEPTQ-135 NMR screening were carried out using $CDCl_3$ and DMSO-d_6 solvents and measured at δ scale. IR was performed using a KBr disc.

2.2. (3-Benzoxazol-2-yl-phenyl)-benzylidine-amine (2a). Solid white colored; yield (84%); mp 173–5 °C; IR: 3054 (CH aromatic) and 1609 (C=N) cm^{-1}; ^{1}HNMR: 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}CNMR: 110.25, 110.55, 11,467, 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 ($C_{20}H_{14}N_2O$): 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-benzylidine) amine (2b). Solid white colored; yield: 82%; mp 183–5 °C; IR: 3053 (CH arom.), 2913 (CH ali.), and 1595 (N=C) cm^{-1}; ^{1}HNMR: 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}CNMR: 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 ($C_{21}H_{16}N_2O$): 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-benzylidine)-amine (2c). Yellow solid colored, yield: 79%; mp 189–91 °C; IR: 3058 (CH arom.) and 1586 (C=N) cm^{-1}; ^{1}HNMR: 7.19–7.40 (m, 6H, benzoxazolyl H4,5,6,7 and florophenyl 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, florophenyl H2,6), and 8.48 (s, 1H, N=CH); ^{13}CNMR; 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 ($C_{20}H_{13}FN_2O$): 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-benzylidine)-amine (2d). White solid colored; yield: 75%; mp 266–8°C; IR: 3047 (CH arom.), 2928(CH aliph.), and 1590 (N=C) cm^{-1}; ^{1}HNMR: 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}CNMR: 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 ($C_{21}H_{16}N_2O_2$): C, 76.81; H, 4.91; N, 8.53; present: C, 76.70; H, 4.90; N, 8.60.

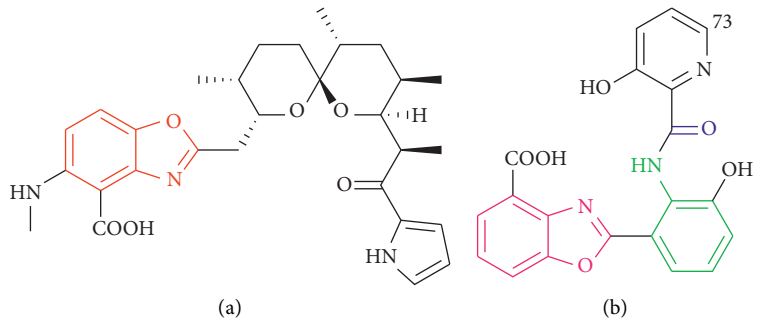


Figure 1: Structures of natural antibiotics containing benzoxazole nucleus.

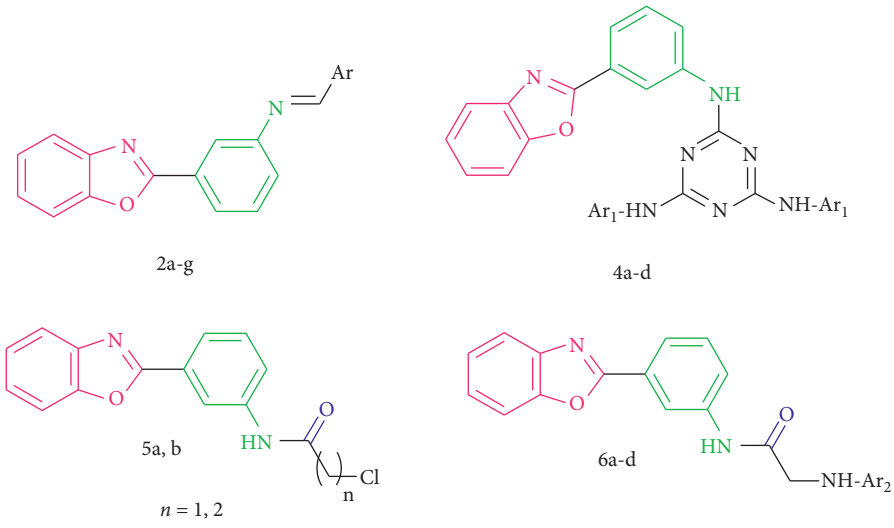


Figure 2: Design of target compounds containing 3-benzoxazol-2ylphenyl moiety.

2.6. (3-Benzoxazol-2-yl-phenyl)-(4-chlorobenzylidine)-amine (2e). Pale yellow crystals; yield: 87%; mp 234–6°C; IR: 3054 (CH arom.) and 1612 (C=N) cm^{-1}; ^{1}HNMR: 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 H3,5) 8.32 (d, $J = 8.4$ Hz, 2H, chlorophenyl H2,6), and 8.49 (s, 1H, N=CH); ^{13}CNMR: 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 ($C_{20}H_{13}ClN_2O$): 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-difluorobenzylidine)-amine (2f). Yellow crystals; yield: 81%; mp 209–11 °C; IR: 3058 (CH arom.) and 1593 (C=C) cm^{-1}; ^{1}HNMR: 7.28–7.40 (m, 5H, benzoxazolyl H4,5,6,7 and florophenyl 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, florophenyl H2,6), and 8.45 (s, 1H, N=CH); ^{13}CNMR: 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 ($C_{20}H_{12}F_2N_2O$): 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-benzylidine)-amine (2g). White crystals; yield: 83%; mp 240–2°C; IR: 3057 (CH arom.) and 1613 (C=N) cm^{-1}; ^{1}HNMR: 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, floromethylphenyl H2,6), and 8.55 (s, 1H, N=CH); ^{13}CNMR: 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 ($C_{21}H_{13}F_3N_2O$): 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}; ^{1}HNMR: 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}CNMR: 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 ($C_{16}H_9Cl_2N_5O$): 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}; ^{1}HNMR: 7.14–7.16 (m, 4H, 2 phenylamino 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}CNMR: 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 ($C_{28}H_{21}N_7O$): 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}; ^{1}HNMR: 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}CNMR: 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 ($C_{30}H_{25}N_7O$): C, 72.13; H, 5.04; N, 19.63; present: C, 72.30; H, 5.00; N, 19.60.

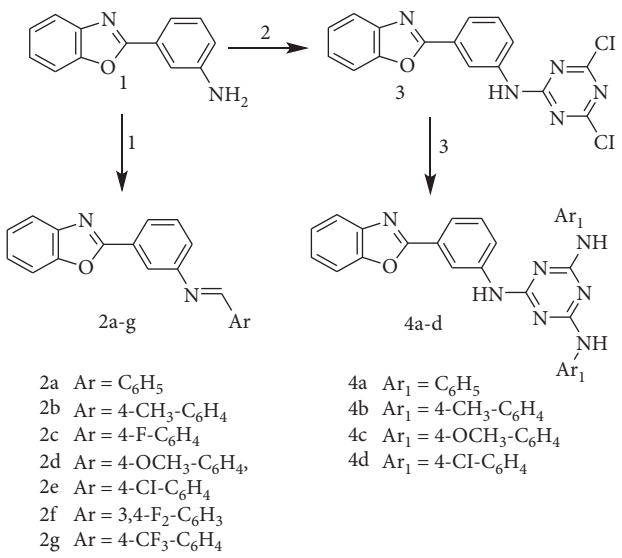
2.13. (3-Benzoxazol-2-yl-phenyl) (4,6-di(4-methoxphenylamino) [1,3,5]triazin-2-yl)amine (4c). Solid Buff colored; yield: 69%; mp 248–50 °C; IR: 3401, 3284 (2NH), 2998 (CH arom.), and 1614 (C=N) cm^{-1}; ^{1}HNMR: 3.34 (s, 6H, two-OCH_3), 6.92 (d, 4H, $J = 8.8$, two-methoxyphenyl H2,6), 7.37–7.41 (m, 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}CNMR: 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 ($C_{30}H_{25}N_7O_3$): 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}; ^{1}HNMR: 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, IH, NH); ^{13}CNMR: 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 ($C_{28}H_{19}Cl_2N_7O$): C, 62.23; H, 3.54; N, 18.14; present: C, 62.20; H, 3.60; N, 18.20.

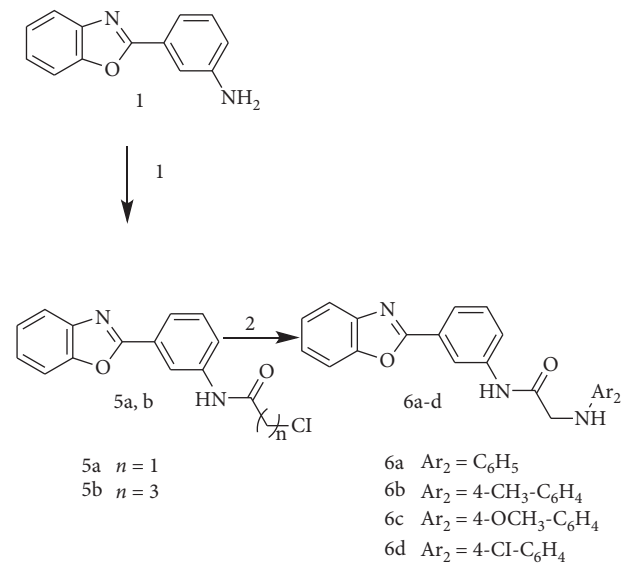
2.15. The General Method of Chloroketones 5a&b Synthesis. To a stirred ice-cold solution of **1** (2.10 g, 0.01 mol) in anhydrous dimethylformamide (10 mL), the appropriate chloroacyl chloride (1.12 g) was added slowly, and then, adding K_2CO_3 (1.38 g) was added. The mixture was further stirred for 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}; ^{1}HNMR: 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}CNMR: 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 ($C_{15}H_{11}ClN_2O_2$): 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}; ^{1}HNMR: 2.03–2.09 (m, 2H, $CH_2CH_2CH_2Cl$), 2.55 (t, 2H, $J = 7.2$, $CH_2CH_2CH_2Cl$), 3.72 (t, 2H, $J = 6.4$, $CH_2CH_2CH_2Cl$), 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}CNMR: 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 ($M^{+\cdot}$+2, 12.54%); calculated analysis of ($C_{17}H_{15}ClN_2O_2$): 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, CH_3CH_2 OH, CH_3COOH, reflux, 4 h; (2) cyanuric chloride, acetone, rt, 1 h; and (3) aryl amine, K_2CO_3, dioxane, reflux, 12 h.



Scheme 2: Synthetic pathway of **5a-b** and **6a–d. Condition and reagents**: (1) acid chloride, K_2CO_3, dimethyl formamide, rt, 24 h; (2) aryl amines, TEA, CH_3CH_2 OH, reflux, 12 h.

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}; ^{1}HNMR: 3.99 (s, 2H, CH_2), 4.36 (s,1H, NH), 6.74 (d, 2H, J = 8 Hz phenylamino, H2,6), 6.90–6.93 (m, 1H, phenylamino H4), 7.28–7.45(m, 6H, benzoxazolyl H4,5,6,7 and phenylamino H3,5), 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); ^{13}CNMR: 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 ($C_{21}H_{17}N_3O_2$): 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}; ^{1}HNMR: 2.29 (s, 3H, CH_3), 3.94 (s, 2H, CH_2), 4.30 (s, 1H, NH), 6.65 (d, 2H, J = 8 Hz, 4-methylphenylamino. H2,6), 7.08 (d, 2H, J = 8 Hz,4-methyphenylamino. H3,5), 7.25–7.58 (m, 4H, benzoxazolyl H4,5,6,7), 7.58 (s, 1H, phenyl H2), 7.74–7.82 (m, 2H, phenyl H4,5), 8.18–828 (m, 2H, phenyl H2,6), and 8.94 (s,1H, NH); ^{13}CNMR: 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 analysis of ($C_{22}H_{19}N_3O_2$): 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}; ^{1}HNMR: 3.63 (s, 3H, OCH_3), 3.87 (s, 2H, CH_2), 5.675–5.68 (s, 1H, NH), 6.58 (d, 2H, J = 8.8 Hz, 4-methoxyphenylamino H2,6), 6.75 (d, 2H, J = 8.8 Hz, 4-methoxyphenylamino H3,5), 7.39–7.42 (m, 2H, benzoxazolyl H5,6), 7.75–7.88 (m, 4H, benzoxazolyl H4,7 and phenyl H4,5), 8.14–8.16 (m, 2H, phenyl H2,6), and 10.31 (s,1H, NH); ^{13}CNMR: 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 (C_{22} $H_{19}N_3O_3$): 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}; ^{1}HNMR: 3.94 (s, 2H, CH_2), 6.29 (s,1H, NH), 6.62 (d, 2H, J = 8.4 Hz, 4-chlorophenylamino H2,6), 7.13 (d, 2H, J = 8.4 Hz, 4-chlorophenylamino H3,5), 7.39–7.41 (m, 2H, benzoxazolyl H5,6), 7.74–7.85 (m, 4H, benzoxazolyl H4,7 and phenyl H4,5), 8.14–8.16 (m, 2H, phenyl H2,6), and 10.43 (s,1H, NH); ^{13}CNMR: 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 ($M^{+\cdot}$+2, 1.77%); calculated analysis of ($C_{21}H_{16}ClN_3O_2$): 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 (**A**merican **T**ype **C**ulture **C**ollection). 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 μL 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 IC_{50} values of 0.214, 0.06, 0.06, 0.10, and 0.01 μM against *M. intracellulare, MRSA, E. coli, St. aureus*, *and P. aeruginosa*, respectively. Amphotericin B exhibited IC_{50} values of 0.17, 0.57, 0.22, 0.17, and 1.30 μM 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 *u*l and two percentage of hematocrit in RPMI 1640 medium supplemented with ten percentage of human serum and sixty μg/ml amikacin) is added to the flat bottom microplates (96-well) containing 10 μl 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 N_2, five percent of O_2, and five percent of CO_2. The Makler and Hinrichs procedure was followed to establish the plasmodial LDH action [36]. For half hour, a mixture of 100 μl of Malstat reagent and twenty μL of the incubation mixture were incubated at 25°C. 20 μL 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 μl of five percent of CH_3COOH, 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, DSMO 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 IC_{50} 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 1×10^6 cells/ml for promastigote assays. A *Leishmania donovani* axenic amastigotes three-day-old culture was diluted with the same medium to 2×10^6 cells/ml for axenic amastigote assays. In the exponential phase, *Trypanasoma 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 *Trypanasoma brucei* trypamastigote, *Leishmania donovani* axenic amastigote, and *Leishmania donovani* promastigote cultures. All samples have been assessed at three serial dilutions starting from 10 μg/mL to 0.4 μg/mL. At 26°C for 72 hours (37°C for *Trypanasoma brucei* trypamastigotes 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 CO_2 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 CO_2 incubator. Alamar Blue assessment was followed for determination of the cell growth, and the IC_{90} data were calculated from the dose-response curves.

2.26. Molecular Modeling Study. The protein structure for *Pf*PNP 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 a 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 chlorobutyryl 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), *Staphylococus 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 μg/mL concentration, except 2-chloroacetamide **5a**, with an IC_{50} 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 antiprotozoal 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 IC_{50} values of 5.1 and 2.2 μM, respectively. Compound **6d** showed comparable antimalarial activities to compound **5a** against the both strains with IC_{50} 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 IC_{50} values of 17.9 and 23.7 μM, respectively, along with

Table 1: Antimalarial action of the potential derivatives.

Compound	*P. falciparum*, D6		*P. falciparum*, 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) (IC_{50} for Vero cells/IC_{50} for *P. falciparum*).

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

Comp.	*L. donovani* promastigote		*L. donovani* amastigote		*L. donovani* Amastigote + THP		*T. brucei*		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

[a]IC_{50}: concentration causing 50% growth inhibition; [b]IC_{90}: concentration causing 90% growth inhibition; Amb = amphotericin B; Pm = pentamidine; DFMO = difluoromethylornithine.

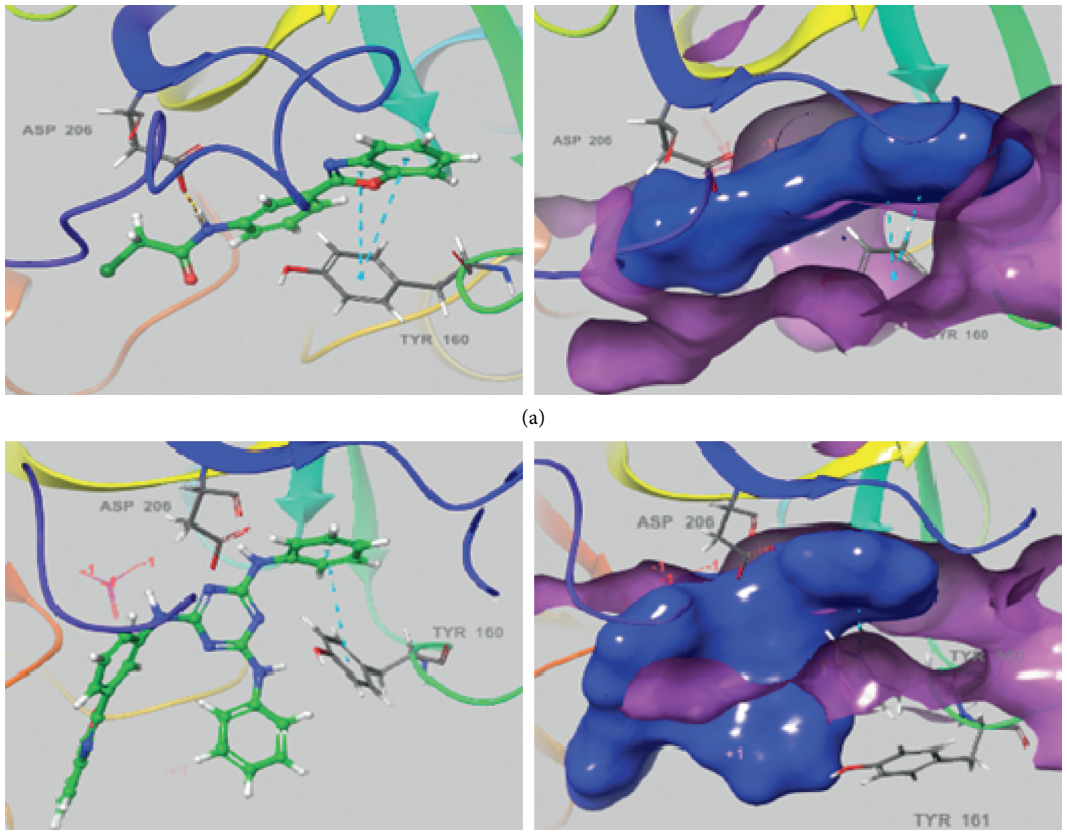


Figure 3: Binding modes of compounds **5a** (a) and **4a** (b) into the PfPNP active site; along with the interacting amino acids represented as labeled tube models, in a ball and stick model (left), the ligands are depicted. The binding cavities' channel surfaces are presented in purple, and the ligand 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 PfPNP *(P. falciparum* purine nucleoside phosphorylase), the key enzyme in purine recycling and salvage. Inhibition of PfPNP 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]. PfPNP catalyzes inosine phosphorolysis into ribose-1-phosphate and hypoxanthine, the primary purine substrate for the salvage pathway [53]. Therefore, we considered PfPNP as a molecular target of the proposed synthesized compounds. The crystal structure of PfPNP is available in the protein data bank (PDB) with PDB ID of 3PHC [54]. The crystal structure of PfPNP 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 PfPNP 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 antiprotozoal 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.

Acknowledgments

The authors extend their appreciation to the Deanship of Scientific Research at Jouf University for funding this work through research Grant No. DSR2020-04-509. This research was also funded by the Deanship of Scientific Research at Princess Nourah Bint Abdulrahman University through the Fast-track Research Funding Program. The authors would like to express their special appreciation and thanks to their colleague Dr. Bijo Mathew, who had been a great research collaborator for them.

Supplementary Materials

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

References

[1] WHO, *Who Fact Sheet on Malaria. Fact Sheet no 94*, World Health Organization, Geneva, Switzerland, 2007.

[2] S. I. Hay, C. A. Guerra, A. J. Tatem, P. M. Atkinson, and R. W. Snow, "Urbanization, malaria transmission and disease burden in Africa," *Nature Reviews Microbiology*, vol. 3, no. 1, p. 81, 2005.

[3] R. Kerb, R. Fux, K. Mörike et al., "Pharmacogenetics of antimalarial drugs: effect on metabolism and transport," *The Lancet Infectious Diseases*, vol. 9, no. 12, pp. 760–774, 2009.

[4] A. P. Phyo, S. Nkhoma, K. Stepniewska et al., "Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study," *The Lancet*, vol. 379, no. 9830, pp. 1960–1966, 2012.

[5] J. A. Peixoto, M. L. Andrade e Silva, A. E. M. Crotti et al., "Antileishmanial activity of the hydroalcoholic extract of miconia langsdorffii, isolated compounds, and semi-synthetic derivatives," *Molecules*, vol. 16, no. 2, pp. 1825–1833, 2011.

[6] S. K. Tipparaju, S. Joyasawal, M. Pieroni, M. Kaiser, R. Brun, and A. P. Kozikowski, "In pursuit of natural product leads: synthesis and biological evaluation of 2-[3-hydroxy-2-[(3-hydroxypyridine-2-carbonyl)amino]phenyl]benzoxazole-4-carboxylic acid (A-33853) and its analogues: discovery ofN-(2-Benzoxazol-2-ylphenyl)benzamides as novel anti-leishmanial chemotypes," *Journal of Medicinal Chemistry*, vol. 51, no. 23, pp. 7344–7347, 2008.

[7] D. Abbanat, M. Macielag, and K. Bush, "Novel antibacterial agents for the treatment of serious gram-positive infections," *Expert Opinion on Investigational Drugs*, vol. 12, no. 3, pp. 379–399, 2003.

[8] D. M. Livermore, "Antibiotic resistance in staphylococci," *International Journal of Antimicrobial Agents*, vol. 16, no. Suppl 1, pp. S3–S10, 2000.

[9] K. Poole, "Multidrug resistance in gram-negative bacteria," *Current Opinion in Microbiology*, vol. 4, no. 5, pp. 500–508, 2001.

[10] İ Yalçin, İ Ören, E. Şener, A. Akin, and N. Uçartürk, "The synthesis and the structure-activity relationships of some substituted benzoxazoles, oxazolo(4, 5-b)pyridines, benzothiazoles and benzimidazoles as antimicrobial agents," *European Journal of Medicinal Chemistry*, vol. 27, no. 4, pp. 401–406, 1992.

[11] D. Díez-Martin, N. R. Kotecha, S. V. Ley et al., "Total synthesis of the ionophore antibiotic cp-61,405 (routiennocin)," *Tetrahedron*, vol. 48, no. 37, pp. 7899–7938, 1992.

[12] I. Oren, O. Temiz, I. Yalçin, E. Sener, A. Akin, and N. Uçartürk, "Synthesis and microbiological activity of 5(or 6)-methyl-2-substituted benzoxazole and benzimidazole derivatives," *Arzneimittel-Forschung*, vol. 47, no. 12, pp. 1393–1397, 1997.

[13] M. M. Al-Sanea, A. Z. Abdelazem, B. S. Park et al., "Ros1 kinase inhibitors for molecular-targeted therapies," *Current Medicinal Chemistry*, vol. 23, no. 2, pp. 142–160, 2016.

[14] S. S. R. Alsayed, H. A. H. Elshemy, M. A. Abdelgawad, M. S. Abdel-Latif, and K. R. A. Abdellatif, "Design, synthesis and biological screening of some novel celecoxib and etoricoxib analogs with promising cox-2 selectivity, anti-inflammatory activity and gastric safety profile," *Bioorganic Chemistry*, vol. 70, pp. 173–183, 2017.

[15] A. Akbay, I. Ören, O. Temiz-Arpaci, E. Aki-Sener, and I. Yalçin, "Synthesis and HIV-1 reverse transcriptase inhibitor activity of some 2,5,6-substituted benzoxazole, benzimidazole, benzothiazole and oxazolo(4,5-b)pyridine derivatives," *Arzneimittel-Forschung*, vol. 53, no. 4, pp. 266–271, 2003.

[16] R. K. Plemper, K. J. Erlandson, A. S. Lakdawala et al., "A target site for template-based design of measles virus entry inhibitors," *Proceedings of the National Academy of Sciences*, vol. 101, no. 15, pp. 5628–5633, 2004.

[17] I. Yildiz-Oren, B. Tekiner-Gulbas, I. Yalcin, O. Temiz-Arpaci, E. Akı-Sener, and N. Altanlar, "Synthesis and antimicrobial activity of new 2-[p-substituted-benzyl]-5-[substituted-carbonylamino]benzoxazoles," *Archiv der Pharmazie*, vol. 337, no. 7, pp. 402–410, 2004.

[18] A. Pinar, P. Yurdakul, I. Yildiz et al., "Some fused heterocyclic compounds as eukaryotic topoisomerase ii inhibitors," *Biochemical and Biophysical Research Communications*, vol. 317, no. 2, pp. 670–674, 2004.

[19] O. Temiz-Arpaci, B. Tekiner-Gulbas, I. Yildiz, E. Aki-Sener, and I. Yalcin, "3d-qsar analysis on benzazole derivatives as eukaryotic topoisomerase ii inhibitors by using comparative molecular field analysis method," *Bioorganic & Medicinal Chemistry*, vol. 13, no. 23, pp. 6354–6359, 2005.

[20] B. Tekiner-Gulbas, O. Temiz-Arpaci, I. Yildiz, E. Aki-Sener, and I. Yalcin, "3d-qsar study on heterocyclic topoisomerase ii inhibitors using comsia," *SAR and QSAR in Environmental Research*, vol. 17, no. 2, pp. 121–132, 2006.

[21] H. Lage, E. Aki-Sener, and I. Yalcin, "High antineoplastic activity of new heterocyclic compounds in cancer cells with resistance against classical DNA topoisomerase ii-targeting drugs," *International Journal of Cancer*, vol. 119, no. 1, pp. 213–220, 2006.

[22] M. A. Abdelgawad, A. Belal, and O. M. Ahmed, "Synthesis, molecular docking studies and cytotoxic screening of certain novel thiazolidinone derivatives substituted with benzothiazole or benzoxazole," *Journal of Chemical and Pharmaceutical Research*, vol. 5, pp. 318–327, 2013.

[23] K. R. A. Abdellatif, E. K. A. Abdelall, M. A. Abdelgawad, D. M. E. Amin, and H. A. Omar, "Design, synthesis and biological evaluation of new 4-(4-substituted-anilino)quinoline derivatives as anticancer agents," *Medicinal Chemistry Research*, vol. 26, no. 5, pp. 929–939, 2017.

[24] M. M. Kandeel, S. M. Ali, E. Elall, M. A. Abdelgawad, and P. F. Lamie, "Synthesis and antitumor activity of novel pyrazolo [3, 4-d] pyrimidines and related heterocycles," *Der Pharma Chemica*, vol. 4, pp. 1704–1715, 2012.

[25] K. R. A. Abdellatif, M. A. Abdelgawad, M. B. Labib, and T. H. Zidan, "Synthesis and biological evaluation of new diarylpyrazole and triarylimidazoline derivatives as selective COX-2 inhibitors," *Archiv der Pharmazie*, vol. 350, no. 8, Article ID 1600386, 2017.

[26] K. R. A. Abdellatif, M. A. Abdelgawad, H. A. H. Elshemy, S. S. R. Alsayed, and G. Kamel, "Synthesis and anti-inflammatory evaluation of new 1,3,5-triaryl-4,5-dihydro-1h-pyrazole derivatives possessing an aminosulphonyl pharmacophore," *Archives of Pharmacal Research*, vol. 38, no. 11, pp. 1932–1942, 2015.

[27] B. Tekiner-Gulbas, O. Temiz-Arpaci, I. Yildiz, and N. Altanlar, "Synthesis and in vitro antimicrobial activity of new 2-[p-substituted-benzyl]-5-[substituted-carbonylamino]benzoxazoles," *European Journal of Medicinal Chemistry*, vol. 42, no. 10, pp. 1293–1299, 2007.

[28] R. Prajapat, B. Soni, A. Bhandari, L. Soni, and S. Kaskhedikar, "Qsar modeling of benzoxazole derivatives as antimicrobial agents," *Der Pharmacia Lettre*, vol. 3, pp. 161–170, 2011.

[29] M. A. Abdelgawad, A. Belal, H. A. Omar, L. Hegazy, and M. E. Rateb, "Synthesis, anti-breast cancer activity, and molecular modeling of some benzothiazole and benzoxazole derivatives," *Archiv der Pharmazie*, vol. 346, no. 7, pp. 534–541, 2013.

[30] M. A. Abdelgawad, K. R. Abdellatif, and O. M. Ahmed, "Design, synthesis and anticancer screening of novel pyrazole derivatives linking to benzimidazole, benzoxazole and benzothiazole," *Medicinal Chemistry*, vol. S1, pp. 2161–0444, 2014.

[31] A. K. Oraby, K. R. A. Abdellatif, M. A. Abdelgawad, K. M. Attia, L. N. Dawe, and P. E. Georghiou, "2,4-Disubstituted phenylhydrazonopyrazolone and isoxazolone derivatives as antibacterial agents: synthesis, preliminary biological evaluation and docking studies," *ChemistrySelect*, vol. 3, no. 11, pp. 3295–3301, 2018.

[32] M. A. Abdelgawad, R. B. Bakr, W. Ahmad, M. M. Al-Sanea, and H. A. H. Elshemy, "New pyrimidine-benzoxazole/benzimidazole hybrids: synthesis, antioxidant, cytotoxic activity, in vitro cyclooxygenase and phospholipase a2-v inhibition," *Bioorganic Chemistry*, vol. 92, Article ID 103218, 2019.

[33] M. M. Al-Sanea, A. Elkamhawy, S. Paik et al., "Sulfonamide-based 4-anilinoquinoline derivatives as novel dual aurora kinase (aurka/b) inhibitors: synthesis, biological evaluation and in silico insights," *Bioorganic & Medicinal Chemistry*, vol. 28, no. 13, Article ID 115525, 2020.

[34] A. Solankee, G. Patel, and K. Patel, "Antibacterial evaluation of some novel 5-imidazolones," *NISCAIR Journal*, vol. 50B, no. 07, 2011.

[35] M. A. Wikler, "Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard," *CLSI (NCCLS)*, vol. 26, pp. M7–A7, 2006.

[36] S. G. Franzblau, R. S. Witzig, J. C. McLaughlin et al., "Rapid, low-technology mic determination with clinical mycobacterium tuberculosis isolates by using the microplate alamar blue

assay," *Journal of Clinical Microbiology*, vol. 36, no. 2, pp. 362–366, 1998.

[37] R. Piper, S. Houzé, L. Wentworth et al., "Immunocapture diagnostic assays for malaria using plasmodium lactate dehydrogenase (pldh)," *The American Journal of Tropical Medicine and Hygiene*, vol. 60, no. 1, pp. 109–118, 1999.

[38] A. Rahman, V. Samoylenko, M. Jacob et al., "Antiparasitic and antimicrobial indolizidines from the leaves ofProsopis glandulosavar.glandulosa," *Planta Medica*, vol. 77, no. 14, pp. 1639–1643, 2011.

[39] S. Jain, M. Jacob, L. Walker, and B. Tekwani, "Screening north american plant extracts in vitro against trypanosoma brucei for discovery of new antitrypanosomal drug leads," *BMC Complementary Medicine and Therapies*, vol. 16, p. 131, 2016.

[40] S. K. Jain, R. Sahu, L. A. Walker, and B. L. Tekwani, "A parasite rescue and transformation assay for antileishmanial screening against intracellular leishmania donovani amastigotes in thp1 human acute monocytic leukemia cell line," *Journal of Visualized Experiments*, vol. 70, Article ID e4054, 2012.

[41] W. Shi, L.-M. Ting, G. A. Kicska et al., "Plasmodium falciparum purine nucleoside phosphorylase," *Journal of Biological Chemistry*, vol. 279, no. 18, pp. 18103–18106, 2004.

[42] P. D. Bank, "Protein data bank," *Nature New Biology*, vol. 233, p. 223, 1971.

[43] J. Du, H. Sun, L. Xi et al., "Molecular modeling study of checkpoint kinase 1 inhibitors by multiple docking strategies and prime/MM-GBSA calculation," *Journal of Computational Chemistry*, vol. 32, no. 13, pp. 2800–2809, 2011.

[44] Schrödinger, LLC, *Release, S4: Ligprep*, Schrödinger, LLC, New York, NY, USA, 2017.

[45] I.-J. Chen and N. Foloppe, "Drug-like bioactive structures and conformational coverage with the ligprep/confgen suite: comparison to programs moe and catalyst," *Journal of Chemical Information and Modeling*, vol. 50, no. 5, pp. 822–839, 2010.

[46] J. R. Greenwood, D. Calkins, A. P. Sullivan, and J. C. Shelley, "Towards the comprehensive, rapid, and accurate prediction of the favorable tautomeric states of drug-like molecules in aqueous solution," *Journal of Computer-Aided Molecular Design*, vol. 24, no. 6-7, pp. 591–604, 2010.

[47] M. P. Repasky, M. Shelley, and R. A. Friesner, "Flexible ligand docking with glide," *Current Protocols in Bioinformatics*, vol. 18, pp. 8.12. 11–18.12. 36, 2007.

[48] A. Solankee and R. Patel, "Synthesis of some novel chalcones, pyrazolines, aminopyrimidines and their antimicrobial study," *Indian Journal of Chemistry Section B*, vol. 53, no. 11, pp. 1448–1453, 2014.

[49] M. Al-Sanea, A. Elkamhawy, A. Zakaria et al., "Synthesis and in vitro screening of phenylbipyridinylpyrazole derivatives as potential antiproliferative agents," *Molecules*, vol. 20, no. 1, pp. 1031–1045, 2015.

[50] M. M. Al-Sanea, B. S. Park, A. Z. Abdelazem et al., "Optimization of bipyridinyl pyrazole scaffolds via design, synthesis and screening of a new series of ros1 kinase-modulating compounds," *Bulletin of the Korean Chemical Society*, vol. 36, pp. 305–311, 2015.

[51] M. Al-Sanea, L. Gotina, F.A. Mohamed et al., "Design, synthesis and biological evaluation of new hdac1 and hdac2 inhibitors endowed with ligustrazine as a novel cap moiety," *Drug Design, Development and Therapy*, vol. 14, p. 497, 2020.

[52] M. M. Al-Sanea, D. G. T. Parambi, M. E. Shaker et al., "Design, synthesis, and in vitro cytotoxic activity of certain 2-[3-Phenyl-4-(pyrimidin-4-yl)-1H-pyrazol1-yl]acetamide derivatives," *Russian Journal of Organic Chemistry*, vol. 56, no. 3, pp. 514–520, 2020.

[53] B. Aneja, B. Kumar, M. A. Jairajpuri, and M. Abid, "A structure guided drug-discovery approach towards identification of plasmodium inhibitors," *RSC Advances*, vol. 6, no. 22, pp. 18364–18406, 2016.

[54] N. Deshpande, K. J. Addess, W. F. Bluhm et al., "The rcsb protein data bank: a redesigned query system and relational database based on the mmcif schema," *Nucleic Acids Research*, vol. 33, pp. D233–D237, 2005.

[55] T. Cheviet, I. Lefebvre-Tournier, S. Wein, and S. Peyrottes, "Plasmodium purine metabolism and its inhibition by nucleoside and nucleotide analogues," *Journal of Medicinal Chemistry*, vol. 62, no. 18, pp. 8365–8391, 2019.

[56] S. Tahlan, S. Kumar, K. Ramasamy et al., "In-silico molecular design of heterocyclic benzimidazole scaffolds as prospective anticancer agents," *BMC Chemistry*, vol. 13, p. 90, 2019.