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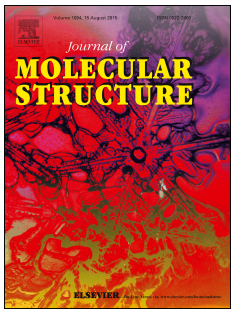

Novel substituted benzothiazole and Imidazo[2,1-*b*][1,3,4]Thiadiazole derivatives: Synthesis, characterization, molecular docking study, and investigation of their *in vitro* antileishmanial and antibacterial activities

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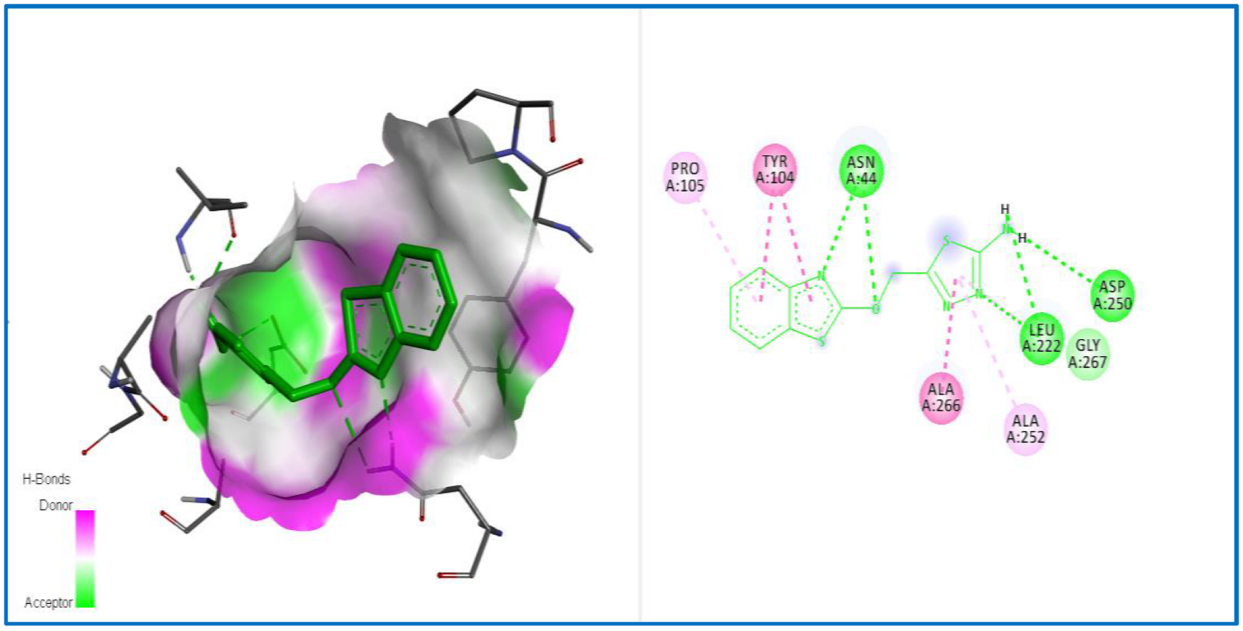

Novel Substituted Benzothiazole and Imidazo[2,1-*b*][1,3,4]Thiadiazole Derivatives: Synthesis, Characterization, Molecular Docking Study, and Investigation of Their *In Vitro* Antileishmanial and Antibacterial Activities

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Abstract

In this study, we synthesized new imidazo[2,1-*b*][1,3,4]thiadiazole derivatives containing benzothiazole group. To this end, we firstly obtained the benzo[*d*]thiazol-2-ylthio/oxy acetonitrile compounds (**3a,b**), the starting materials, in high yields (82% and 87%, respectively). Then, we synthesized the 2-amino-1, 3,4-thiadiazole derivatives **(4a,b)** from the reaction of these nitrile derivatives **(3a,b)** with thiosemicarbazide in trifluoroacetic acid (TFA) (in yields of 83% and 84%). Finally, we synthesized the imidazo[2,1-*b*][1,3,4]thiadiazole derivatives **(5-24)** containing benzothiazole group, which are the target compounds, from reactions of 2-amino-1,3,4-thiadiazole derivatives **(4a,b)** with phenacyl bromide derivatives (in yields of 53% to 73%).

All of the compounds synthesized were characterized with ^{1}H NMR, ^{13}C NMR, FT-IR, elemental analysis, and mass spectroscopy.

Antileishmanial and antibacterial activity tests were applied to the compounds synthesized in the study. It was observed that compound **8** had the highest antileishmanial activity (MIC=10 000 μg/mL). Also, compounds **7** and **17** were found to be effective at the highest concentration studied (MIC=20 000 μg/mL). In terms of antibacterial activity, compounds **4b** and **7** were found to be the most effective compounds against *Escherichia coli* (MIC = 625 μg/mL).

Theoretical calculations were performed to support the experimental results. To this end, we performed Molecular Docking studies to determine whether or not the compounds (**4a**, **4b**, **7** and **13)** optimized with Gaussian09 using the DFT/B3LYP/6-31G(d,p) theory, which is a quantum chemical calculation, could be an inhibitor agent for the 2eg7 *Escherichia coli* protein structure. Also, we investigated the relationship between the calculated HOMO values of these four ligands and docking studies.

Keywords: Benzothiazole; Imidazo[2,1-*b*][1,3,4]thiadiazole; Biological activity; Molecular docking; HOMO.

1. Introduction

Antibacterial drugs (antibiotics) kill bacteria and prevent them from reproducing. However, the inappropriate and excessive use of antibiotics causes these microorganisms to develop resistance against these substances. Also, the constant use of antibiotics transforms health bacteria into harmful microorganisms over time. Various active ingredients that exhibit antibacterial activity is being developed as technology advances, leading to the availability of more drugs that are able to treat various bacterial infections [1].

Leishmania, or more commonly known as oriental sores, is a serious health problem. Antileishmanial drugs kill Leishmania protozoa and prevent their fatal disease.

Benzothiazoles, thiadiazoles, and imidazo[2,1-*b*][1,3,4]thiadiazoles constitute an important class in heterocyclic chemistry due to their wide spectrum of biological activities. Various active ingredients containing these compounds were synthesized in a laboratory environment and were eventually introduced to be used to treat various diseases [1,2].

A number of studies have shown that imidazo[2,1-*b*][1,3,4]thiadiazole derivatives have various antimicrobial, antifungal, anticancer, antituberculosis, anti-inflammatory, anticonvulsant, analgesic, anesthetic, antihyperlipidemic, and diuretic activities [3-10]. Numerous chemical and medical studies were conducted on imidazo[2,1-*b*][1,3,4]thiadiazole derivatives. These compounds have become a matter of interest in pharmaceutical industry [11-17].

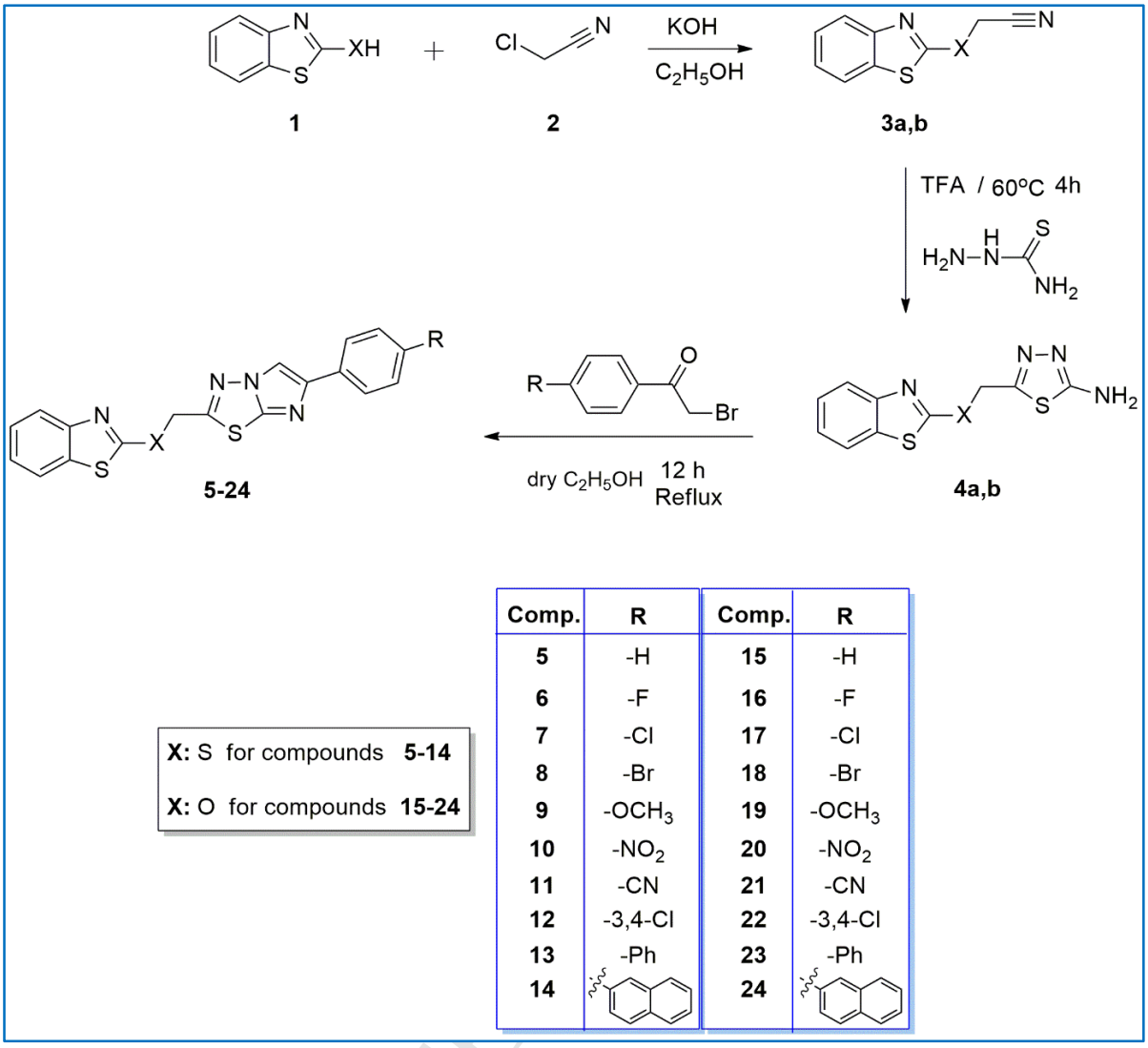
Levamisole is used to regulate and strengthen the immune system. Levamisole and Dexamisole are both enantiomers of Levamisole [18,19], imidazo[2,1-*b*][1,3,4]thiadiazole derivatives, and have attracted the attention of researchers studying anticancer activities [20-

26]. Moreover, imidazo[2,1-*b*][1,3,4]thiadiazoles and their heterocyclic derivatives were reported to be used in a variety of industrial purposes such as paint production [27].

In light of the aforementioned data, this study aims to synthesize compounds containing benzothiazole and imidazo[2,1-*b*][1,3,4]thiadiazole derivatives together **(5-24)**, characterize them, and investigate their antileishmanial and antibacterial activities.

This study also aims to find the optimal structures of the selected compounds **(4a**, **4b**, **7**, and **13)** using the DFT (B3LYP) theory, with the 6-31G(d,p) basis set [28,29] on Gaussian09 [30] in order to determine whether or not these compounds might be possible inhibitor agents for the 2eg7 *Escherichia coli* protein structure using molecular docking simulation.

The synthetic route used to synthesize compounds containing benzothiazole and imidazo[2,1-*b*][1,3,4]thiadiazole derivatives together **(5-24)** is shown in Scheme 1.



Comp.	R	Comp.	R
5	-H	15	-H
6	-F	16	-F
7	-Cl	17	-Cl
8	-Br	18	-Br
9	$-OCH_3$	19	$-OCH_3$
10	$-NO_2$	20	$-NO_2$
11	-CN	21	-CN
12	-3,4-Cl	22	-3,4-Cl
13	-Ph	23	-Ph
14		24	

Scheme 1. Synthetic route for the synthesis of target compounds **(5-24)**.

2. Experimental Section

2.1. General Methods

The ^{1}H NMR and ^{13}C NMR spectra of the compounds were assessed using an Agilent Annual Refill (400 MHz) device. The ESI (+) method and a Thermo TSQ Quantum Access device were used to determine mass spectrum. The IR spectra of the original compounds were calculated using ATR and a PerkinElmer Infrared Spectroscopy (FT-IR) device. Elemental analyses were performed using a LECO 932 CHNS (Leco-932, St. Joseph, MI, USA)

instrument, and the results were within ± 0.4% of the theoretical values. The melting points of the compounds were determined using a Thermo Scientific IA9000 device.

2.2. Synthesis

2.2.1. General procedure for the synthesis of 2-(benzo[d]thiazol-2-ylthio or -2yl-oxy)acetonitrile ***(3a,b)***

Benzo[*d*]thiazol-2-thiol/ol **(1)** (16.72 g/15.12 g; 0.10 mol) and potassium hydroxide (5.61 g, 0.10 mol) were dissolved within ethyl alcohol in a two-necked flask. 2-chloroacetonitrile (7.55 g; 0.10 mol) was added into the reaction mixture. The reaction mixture was refluxed for 5 hours. The resulting product was filtered through a sintered funnel. The filtrate was crystallized using an appropriate solvent and the pure substance was dried with P_2O_5 in A vacuum oven. Finally, structure of the synthesized compound was characterized with FT-IR, ^{1}H NMR, ^{13}C NMR, elemental analysis, and mass spectroscopy.

2.2.1.1. 2-(Benzo[d]thiazol-2-ylthio)acetonitrile ***(3a)***

Light yellow solid, yield:16.97 g (82%), m.p. 74-75 ºC (from DMF-EtOH, 1:5). IR (ATR, cm^{-1}): 3055 (Ar-CH), 2970 (Aliph. CH), 2249 (CN), 1617 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.54 (s, 2H, -CH_2), Ar-H [8.06 (d, *J*=8.0 Hz, 1H), 7.93 (d, *J*=8.4 Hz, 1H), 7.50 (t, *J*=6.8, 8.0 Hz, 1H), 7.41 (t, *J*=7.2, 7.6 Hz, 1H)]. ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 18.55 (-CH_2), Ar-C [122.00 (CH), 122.61 (CH), 125.49 (CH), 127.08 (CH), 135.62 (C), 152.67 (C), 163.74 (C)], 117.83 (CN); MS: *m/z* 209.90 (M+3, 39). *Anal*. Calcd. for ($C_9H_6N_2S_2$): C, 52.40; H, 2.93; N, 13.58. Found: C, 52.43; H, 2.89; N, 13.63.

2.2.1.2. 2-(Benzo[d]thiazol-2yl-oxy)acetonitrile ***(3b)***

Yellowish solid, yield:16.59 g (87%), m.p. 69-70 ºC (DMF-EtOH, 1:6). IR (ATR, cm^{-1}): 3034 (Ar-CH), 2981 (Aliph. CH), 2263 (CN), 1665 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.83 (s, 2H, -CH_2), Ar-H [7.66 (d, *J*=8.0 Hz, 1H), 7.31 (m, 2H), 7.20 (m, 1H)]. ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 61.84 (-CH_2), Ar-C [121.55 (CH), 122.61 (CH), 125.56 (CH), 127.13 (CH), 135.62 (C), 157.82 (C), 170.43 (C)], 115.69 (CN); MS: *m/z* 192.12 (M+2, 5). *Anal*. Calcd. for ($C_9H_6N_2OS$): C, 56.83; H, 3.18; N, 14.73. Found: C, 56.86; H, 3.20; N, 14.68.

2.2.2. General procedure for the synthesis of 5-((benzo[d]thiazol-2-ylthio or -2yl-oxy)methyl)-1,3,4-thiadiazol-2-amine ***(4a,b)***

The **3a** or **3b** compound (0.075 mol) was dissolved within 40 ml trifluoroacetic acid in a round-bottom flask and then thiosemicarbazide (0.100 mol) was added. The mixture was refluxed for 4 hours at 60 °C. The reaction mixture was then poured on 200 ml ice-water mixture and neutralized with diluted ammonia. The resulting substance was filtered through a funnel. The solid substance obtained was washed with water, ethyl alcohol, and diethyl ether, respectively. Afterwards, the substance was purified by crystallization with an appropriate solvent or solvent pair. The pure substance was dried in a P_2O_5 vacuum oven. Finally, structures of the synthesized compounds were characterized with FT-IR, ^{1}H NMR, ^{13}C NMR, elemental analysis, and mass spectroscopy.

2.2.2.1. 5-((Benzo[d]tiyazol-2-iltiyo)metil)-1,3,4-tiyadiazol-2-amin ***(4a)***

Light brown solid, yield: 17.39 g (83%), m.p. 199-201 ºC (from DMF-EtOH, 1:3). IR (ATR, cm^{-1}): 3254-3089 (-NH_2), 3067 (Ar-CH), 2958 (Aliph. CH), 1626 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.81 (s, 2H, -CH_2), Ar-H [8.03 (d, *J*=8.0 Hz, 1H), 7.89 (d, *J*=8.0 Hz, 1H),

7.48 (t, *J*=7.6, 7.6 Hz, 1H), 7.38 (t, *J*=7.2, 7.6 Hz, 1H)], 7.14 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 31.75 (-CH_2), Ar-C [121.74 (CH), 122.46 (CH), 125.22 (CH), 126.96 (CH), 135.48 (C), 152.76 (C), 170.08 (C)], Thiadiazole-C [154.48 (C), 165.49 (C)]; MS: *m/z* 280.79 (M+2, 34). *Anal*. Calcd. for ($C_{10}H_8N_4S_3$): C, 42.84; H, 2.88; N, 19.98. Found: C, 42.81; H, 2.86; N, 19.96.

2.2.2.2. 5-((Benzo[d]tiyazol-2-iloksi)metil)-1,3,4-tiyadiazol-2-amin ***(4b)***

Light brown crystal, yield: 16.55 g (84%), m.p. 238-240 ºC (from DMF-EtOH, 1:2). IR (ATR, cm^{-1}): 3271-3158 (-NH_2), 3035 (Ar-CH), 2979 (Aliph. CH), 1667 (C=N). ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 5.32 (s, 2H, -CH_2), Ar-H [7.66 (d, *J*=8.4 Hz, 1H), 7.37 (m, 2H), 7.20 (m, 2H)], 7.24 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 41.13 (-CH_2), Ar-C [112.13 (CH), 121.77 (CH), 123.51 (CH), 124.04 (CH), 127.15 (C), 136.60 (C), 170.15 (C)], Thiadiazole-C [152.14 (C), 169.24 (C)]; MS: *m/z* 261.04 (M-3, 224). *Anal*. Calcd. for ($C_{10}H_8N_4OS_2$): C, 45.44; H, 3.05; N, 21.20. Found: C, 45.49; H, 3.02; N, 21.26.

2.2.3. General procedure for the synthesis of imidazo[2,1-b][1,3,4]thiadiazole derivatives ***(5-24)***

Substituted 2-amino-1,3,4-thiadiazole derivatives **(4a,b)** (5 mmol) and 2-bromacetophenone derivatives (5 mmol) were dissolved within absolute ethyl alcohol in a two-necked flask. The mixture was refluxed for 12 hours, and the progress of the reaction was checked using thin layer chromatography (TLC). Once the reaction was completed, the mixture was evaporated until almost dry using a rotary evaporator. Then, the reaction mixture was alkalized with diluted Na_2CO_3 solution. The mixture was filtered and washed with water. The substance was purified by crystallized with an appropriate solvent or solvent pair. The pure substance was dried in a P_2O_5 vacuum oven. Lastly, the structures of the synthesized compounds were

characterized with FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectroscopy, and elemental analysis. The physical properties and the spectral data of the products are listed below.

2.2.3.1. 2-((6-Phenylimidazo[2,1-b][1,3,4]thiadiazol-2-yl)methylthio)benzo[d]thiazole ***(5)***

Light brown solid, yield: 1.29 g (68%), m.p.169-170 ºC (from DMF-EtOH, 1:2); IR (ATR, cm^{-1}): 3085 (Ar-CH), 2968 (Aliph. CH), 1601 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.08 (s, 2H, -CH_2), Ar-H [8.02 (d, *J*=8.0 Hz, 1H), 7.90 (d, *J*=8.0 Hz, 1H), 7.82 (d, *J*=6.8 Hz, 2H), 7.48 (t, *J*=8.4 Hz, 1H), 7.37 (m, 3H), 7.24 (t, *J*=7.6, 7.2 Hz, 1H)], Imidazole-H [8.64 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 32.94 (-CH_2), Ar-C [121.84 (CH), 122.55 (CH), 125.13 (CH), 125.38 (CH), 127.05 (CH), 127.90 (CH), 129.09 (CH), 134.20 (C), 135.59 (C), 152.61 (C), 164.91 (C)], Imidazole-C [110.72 (CH), 145.73 (C)], Thiadiazole-C [145.60 (C), 162.40 (C)]; MS: *m/z* 381.07 (M+1, 100). *Anal*. Calcd. for ($C_{18}H_{12}N_4S_3$): C, 56.82; H, 3.18; N, 14.72. Found: C, 56.78; H, 3.21; N, 14.74.

2.2.3.2. 2-((6-(4-Fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)methylthio) benzo[d]thiazole ***(6)***

Light brown solid, yield: 1.18 g (62%), m.p. 184-185 ºC (from Acetone); IR (ATR, cm^{-1}): 3058 (Ar-CH), 2972 (Aliph. CH), 1599 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.08 (s, 2H, -CH_2), Ar-H [8.03 (d, *J*=8.0 Hz, 1H), 7.90 (d, *J*=8.0 Hz, 1H), 7.85 (t, *J*=5.6, 5.6 Hz, 2H), 7.48 (t, *J*=6.4, 7.6 Hz, 1H), 7.38 (t, *J*=7.6, 7.2 Hz, 1H), 7.21 (t, *J*=8.8, 8.0 Hz, 2H)], Imidazole-H [8.63 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 32.91 (-CH_2), Ar-C [115.88 (CH), 121.84 (CH), 122.56 (CH), 125.38 (CH), 127.05 (CH), 130.73 (CH), 135.58 (C), 152.60 (C), 160.77 (C), 162.48 (C), 164.92 (C)], Imidazole-C [110.58 (CH), 145.60 (C)], Thiadiazole-C [144.79(C), 163.20 (C)]; MS: *m/z* 398.72 (M^+, 15). *Anal*. Calcd. for ($C_{18}H_{11}FN_4S_3$): C, 54.25; H, 2.78; N, 14.06. Found: C, 54.31; H, 2.81; N, 14.09.

2.2.3.3. 2-((6-(4-Chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)methylthio) benzo[d]thiazole ***(7)***

White solid, yield: 1,22 g (59%), m.p. 225-226 ºC (from DMF-EtOH, 1:2); IR (ATR, cm^{-1}): 3104 (Ar-CH), 2969 (Aliph. CH), 1623 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.94 (s, 2H, -CH_2), Ar-H [8.07 (d, *J*=8.0 Hz, 1H), 8.01 (d, *J*=8.0 Hz, 2H), 7.93 (d, *J*=8.0 Hz, 1H), 7.69 (d, *J*=8.0 Hz, 2H), 7.52 (t, *J*=7.6, 7.2 Hz, 1H), 7.42 (t, *J*=7.2, 7.6 Hz, 1H)], Imidazole-H [8.81 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 31.43 (-CH_2), Ar-C [113.83 (CH), 121.75 (CH), 122.72 (CH), 125.53 (CH), 127.12 (CH), 129.57 (CH), 130.79 (C), 132.76 (C), 135.88 (C), 155.47 (C), 169.17 (C)], Imidazole-C [111.93 (CH), 152.80 (C)], Thiadiazole-C [139.88 (C), 165.17 (C)]; MS: *m/z* 432.81 (M^+ + H_2O, 100). *Anal*. Calcd. for ($C_{18}H_{11}ClN_4S_3$): C, 52.10; H, 2.67; N, 13.50. Found: C, 52.16; H, 2.71; N, 13.54.

2.2.3.4. 2-((6-(4-Bromophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)methylthio) benzo[d]thiazole ***(8)***

White solid, yield: 1.53 g (67%), m.p. 234-235 ºC (from DMF-EtOH, 1:3); IR (ATR, cm^{-1}): 3089 (Ar-CH), 2873 (Aliph. CH), 1626 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 4.93 (s, 2H, -CH_2), Ar-H [8.07 (d, *J*=7.6 Hz, 1H), 7.93 (d, *J*=7.6 Hz, 3H), 7.84 (d, *J*=8.0 Hz, 2H), 7.53 (t, *J*=7.6, 7.2 Hz, 1H), 7.42 (t, *J*=7.6, 7.2 Hz, 1H)], Imidazole-H [8.91 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 31.40 (-CH_2), Ar-C [112.45 (CH), 121.73 (CH), 122.74 (C), 125.54 (CH), 127.12 (CH), 129.17 (CH), 130.82 (C), 132.53 (CH), 133.06 (C), 152.20 (C), 169.20 (C)], Imidazole-C [111.33 (CH), 148.26 (C)], Thiadiazole-C [135.33 (C), 160.19 (C)]; MS: *m/z* 478.72 (M+1+H_2O, 100). *Anal*. Calcd. for ($C_{18}H_{11}BrN_4S_3$): C, 47.06; H, 2.41; N, 12.20. Found: C, 47.11; H, 2.39; N, 12.22.

2.2.3.5. 2-((6-(4-Methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)methylthio) benzo[d]thiazole ***(9)***

Light yellow solid, yield:1.11 g (54%), m.p. 176-177 ºC (from Acetone); IR (ATR, cm^{-1}): 3136 (Ar-CH), 2976 (Aliph. CH), 1599 (C=N), 1241-1172 (-OCH_3); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.08 (s, 2H, -CH_2), 3.75 (s, 3H, -OCH_3), Ar-H [8.06 (d, *J*=7.6 Hz, 1H), 7.90 (d, *J*=8.0 Hz, 1H), 7.74 (d, *J*=8.0 Hz, 2H), 7.50 (m, 1H), 7.40 (m, 1H)], Imidazole-H [8.51 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 32.91 (-CH_2), 55.58 (-OCH_3), Ar-C [115.83 (CH), 121.83 (CH), 122.56 (CH), 125.78 (CH), 127.00 (C), 130.73 (CH), 135.58 (CH), 152.40 (C), 160.89 (C), 162.48 (C) 164.92 (C)], Imidazole-C [110.58 (CH), 145.64 (C)], Thiadiazole-C [144.76 (C), 163.32 (C)]; MS: *m/z* 411.04 (M+1, 55), 429.10 (M+1+H_2O, 100). *Anal*. Calcd. for ($C_{19}H_{14}N_4OS_3$): C, 55.59; H, 3.44; N, 13.65. Found: C, 55.64; H, 3.47; N, 13.61.

2.2.3.6. 2-((6-(4-Nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)methylthio) benzo[d]thiazole ***(10)***

Yellow solid, yield:1.47 g (69%), m.p. 209-211 ºC (from DMF-EtOH, 1: 1); IR (ATR, cm^{-1}): 3064 (Ar-CH), 2975 (Aliph. CH), 1599 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.10 (s, 2H, -CH_2), Ar-H [8.24 (d, *J*=8.4 Hz, 2H), 8.07 (d, *J*=8.8 Hz, 2H), 8.04 (d, *J*=8.0 Hz, 1H), 7.90 (d, *J*=8.0 Hz, 1H), 7.48 (t, *J*=7.6, 7.2 Hz, 1H), 7.38 (t, *J*=8.0, 7.2 Hz, 1H)], Imidazole-H [8.94 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 32.94 (-CH_2), Ar-C [121.85 (CH), 122.59 (CH), 124.67 (CH), 125.41 (CH), 125.75 (CH), 127.07 (CH), 135.60 (C), 140.74 (C), 146.71 (C), 152.58 (C), 164.90 (C)], Imidazole-C [113.47 (CH), 146.59 (C)], Thiadiazole-C [143.46 (C), 163.81 (C)]; MS: *m/z* 425.79 (M+1, 67). *Anal*. Calcd. for ($C_{18}H_{11}N_5O_2S_3$): C, 50.81; H, 2.61; N, 16.46. Found: C, 50.77; H, 2.59; N, 16.50.

2.2.3.7. 4-(2-((Benzo[d]thiazol-2-ylthio)methyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl) benzonitrile ***(11)***

White solid, yield:1.34 g (66%), m.p. 207-208 ºC (from Acetone); IR (ATR, cm^{-1}): 3068 (Ar-CH), 2998 (Aliph. CH), 2219 (CN), 1608 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.10 (s, 2H, -CH_2), Ar-H [8.03 (d, *J*=8.4 Hz, 1H), 8.00 (d, *J*=7.2 Hz, 2H), 7.90 (d, *J*=8.0 Hz, 1H), 7.84 (d, *J*=8.0 Hz, 2H), 7.49 (t, *J*=7.2, 7.6 Hz, 1H), 7.39 (t, *J*=7.2, 7.6 Hz, 1H)], Imidazole-H [8.88 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 32.93 (-CH_2), Ar-C [109.83 (C), 121.85 (CH), 122.59 (CH), 125.40 (CH), 125.58 (CH), 127.07 (CH), 133.21 (CH), 135.59 (C), 138.70 (C), 152.58 (C), 164.90 (C)], Imidazole-C [112.91 (CH), 146.18 (C)], Thiadiazole-C [143.84 (C), 163.56 (C)], 119.63 (CN); MS: *m/z* 405.70 (M+1, 100). *Anal*. Calcd. for ($C_{19}H_{11}N_5S_3$): C, 56.27; H, 2.73; N, 17.27. Found: C, 56.32; H, 2.75; N, 17.31.

2.2.3.8. 2-((6-(3,4-Dichlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)methylthio) benzo[d]thiazole ***(12)***

Whitish solid, yield:1.59 g (71%), m.p. 197-199 ºC (from DMF-EtOH, 1:2); IR (ATR, cm^{-1}): 3125 (Ar-CH), 2968 (Aliph. CH), 1599 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.09 (s, 2H, -CH_2), Ar-H [8.03 (d, *J*=7.6 Hz, 2H), 7.90 (d, *J*=8.0 Hz, 1H), 7.80 (d, *J*=8.4 Hz, 1H), 7.63 (d, *J*=8.8 Hz, 1H), 7.48 (t, *J*=7.6, 8.0 Hz, 1H), 7.38 (t, *J*=8.4, 8.0 Hz, 1H)], Imidazole-H [8.79 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 32.92 (-CH_2), Ar-C [121.84 (CH), 122.58 (CH), 125.11 (CH), 125.40 (CH), 126.60 (CH), 127.07 (CH), 129.88 (CH), 131.40 (C), 131.99 (C), 134.94 (C), 135.60 (C), 152.59 (C), 164.90 (C)], Imidazole-C [112.00 (CH), 145.73 (C)], Thiadiazole-C [143.18 (C), 163.23 (C)]; MS: *m/z* 448.94 (M+1, 87), 451.00 (M+3, 100). *Anal*. Calcd. for ($C_{18}H_{10}Cl_2N_4S_3$): C, 48.11; H, 2.24; N, 12.48. Found: C, 48.14; H, 2.21; N, 12.53.

2.2.3.9. 2-((6-(4-Phenylphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)methylthio) benzo[d]thiazole ***(13)***

Whitish solid, yield: 1.32 g (58%), m.p. 216-219 ºC (from DMF-EtOH, 1:2); IR (ATR, cm^{-1}): 3102 (Ar-CH), 2947 (Aliph. CH), 1610 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.10 (s, 2H, -CH_2), Ar-H [8.04 (d, *J*=7.2 Hz, 1H), 7.91 (d, *J*=7.2 Hz, 3H), 7.69 (d, *J*=6.0 Hz, 4H), 7.41 (m, 5H)], Imidazole-H [8.71 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 32.87 (-CH_2), Ar-C [121.85 (CH), 122.59 (CH), 125.39 (CH), 125.66 (CH), 126.90 (CH), 127.07 (CH), 127.36 (CH), 127.87 (CH), 129.40 (CH), 133.47 (C), 134.21 (C), 134.79 (C), 135.58 (C), 152.61 (C), 164.92 (C)], Imidazole-C [110.93 (CH), 146.13 (C)], Thiadiazole-C [140.75 (C), 163.10 (C)]; MS: *m/z* 456.80 (M^+, 84). *Anal*. Calcd. for ($C_{24}H_{16}N_4S_3$): C, 63.13; H, 3.53; N, 12.27. Found: C, 63.16; H, 3.49; N, 12.32.

2.2.3.10. 2-((6-(Naphthalen-2-yl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)methylthio) benzo[d]thiazole ***(14)***

Whitish solid, yield: 1.31 g (61%), m.p. 203-205 ºC (from DMF-EtOH, 1:2); IR (ATR, cm^{-1}): 3050 (Ar-CH), 2974 (Aliph. CH), 1598 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.11 (s, 2H, -CH_2), Ar-H [8.36 (s, 1H), 8.04 (d, *J*=7.6 Hz, 1H), 7.97 (d, *J*=7.6 Hz, 1H), 7.91 (m, 4H), 7.48 (m, 3H), 7.38 (t, *J*=8.4, 6.8 Hz, 1H)], Imidazole-H [8.78 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 32.96 (-CH_2), Ar-C [121.86 (CH), 122.59 (CH), 123.24 (CH), 123.85 (CH), 125.40 (CH), 126.28 (CH), 127.08 (CH), 128.09 (CH), 128.38 (CH), 128.64 (C), 131.68 (C), 132.84 (C), 135.69 (C), 152.61 (C), 164.95 (C)], Imidazole-C [111.30 (CH), 145.87 (C)], Thiadiazole-C [133.64 (C), 162.57 (C)]; MS: *m/z* 430.85 (M+1, 100). *Anal*. Calcd. for ($C_{22}H_{14}N_4S_3$): C, 61.37; H, 3.28; N, 13.01. Found: C, 61.39; H, 3.32; N, 12.98.

2.2.3.11. 2-((6-Phenylimidazo[2,1-b][1,3,4]thiadiazol-2-yl)methoxy) benzo[d]thiazole ***(15)***

Yellowish solid, yield: 1.31 g (72%), m.p. 201-203 ºC (from DMF-EtOH, 1:3); IR (ATR, cm^{-1}): 3044 (Ar-CH), 2983 (Aliph. CH), 1601 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.63 (s, 2H, -CH_2), Ar-H [7.81 (d, *J*=7.6 Hz, 2H), 7.69 (d, *J*=7.6 Hz, 1H), 7.46 (d, *J*=7.6 Hz, 1H), 7.37 (m, 3H), 7.24 (m, 2H)], Imidazole-H [8.64 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.24 (-CH_2), Ar-C [111.99 (CH), 121.78 (CH), 123.69 (CH), 124.27 (CH), 125.12 (CH), 127.34 (CH), 127.84 (CH), 129.10 (C), 134.15 (C), 136.48 (C), 169.62 (C)], Imidazole-C [110.96 (CH), 145.88 (C)], Thiadiazole-C [145.19 (C), 159.51 (C)]; MS: *m/z* 364.03 (M^+, 37). *Anal*. Calcd. for ($C_{18}H_{12}N_4OS_2$): C, 59.32; H, 3.32; N, 15.37. Found: C, 59.28; H, 3.35; N, 15.41.

2.2.3.12. 2-((6-(4-Fluorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)methoxy) benzo[d]thiazole ***(16)***

White solid, yield: 1.32 g (69%), m.p. 186-188 ºC (from DMF-EtOH, 1:3); IR (ATR, cm^{-1}): 3105 (Ar-CH), 2973 (Aliph. CH), 1595 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.63 (s, 2H, -CH_2), Ar-H [7.83 (m, 2H), 7.70 (d, *J*=8.0 Hz, 1H), 7.45 (d, *J*=7.6 Hz, 1H), 7.38 (t, *J*=7.6, 7.6 Hz, 1H), 7.23 (m, 3H)], Imidazole-H [8.64 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.24 (-CH_2), Ar-C [111.99 (CH), 116.11 (CH), 121.77 (CH), 123.71 (CH), 127.01 (CH), 127.09 (C), 127.35 (CH), 130.72 (C), 136.48 (C), 160.80 (C), 169.62 (C)], Imidazole-C [110.83 (CH), 145.25 (C)], Thiadiazole-C [144.96(C), 163.23 (C)]; MS: *m/z* 382.95 (M+1, 83). *Anal*. Calcd. for ($C_{18}H_{11}FN_4OS_2$): C, 56.53; H, 2.90; N, 14.65. Found: C, 56.57; H, 2.88; N, 14.69.

2.2.3.13. 2-((6-(4-Chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)methoxy) benzo[d]thiazole ***(17)***

White solid, yield: 1.29 g (65%), m.p. 227-229 °C (from DMF-EtOH, 1:3); IR (ATR, cm^{-1}): 3131 (Ar-CH), 2987 (Aliph. CH), 1588 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.63 (s, 2H, -CH_2), Ar-H [7.81 (d, *J*=8.4 Hz, 2H), 7.70 (d, *J*=8.0 Hz, 1H), 7.42 (m, 4H), 7.23 (t, *J*=7.6, 7.6 Hz, 1H)], Imidazole-H [8.70 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.24 (-CH_2), Ar-C [111.99 (CH), 121.77 (CH), 123.71 (CH), 124.28 (CH), 126.77 (CH), 127.35 (CH), 129.14 (CH),132.21 (C), 133.07 (C), 136.48 (C), 169.63 (C)], Imidazole-C [111.42 (CH), 145.44 (C)], Thiadiazole-C [144.69 (C), 159.83 (C)]; MS: *m/z* 399.05 (M+1, 100). *Anal*. Calcd. for ($C_{18}H_{11}ClN_4OS_2$): C, 54.20; H, 2.78; N, 14.04. Found: C, 54.17; H, 2.81; N, 14.10.

2.2.3.14. 2-((6-(4-Bromophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)methoxy) benzo[d]thiazole ***(18)***

Light yellow solid, yield: 1.42 g (64%), m.p. 241-243 ºC (from DMF-EtOH, 1:3); IR (ATR, cm^{-1}): 3128 (Ar-CH), 2975 (Aliph. CH), 1587 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.63 (s, 2H, -CH_2), Ar-H [7.75 (d, *J*=8.8 Hz, 2H), 7.71 (d, *J*=8.0 Hz, 1H), 7.57 (d, *J*=8.8 Hz, 2H), 7.46 (d, *J*=7.6 Hz, 1H), 7.39 (t, *J*=7.6, 8.0 Hz, 1H), 7.25 (t, *J*=7.6, 7.6 Hz, 1H)], Imidazole-H [8.71 (s, 1H)]; ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 42.25 (-CH_2), Ar-C [112.01 (CH), 120.74 (CH), 121.77 (C), 123.72 (CH), 124.29 (CH), 127.08 (CH), 127.37 (C), 132.06 (CH), 133.43 (C), 136.48 (C), 169.63 (C)], Imidazole-C [111.43 (CH), 145.45 (C)], Thiadiazole-C [144.69 (C), 159.90 (C)]; MS: *m/z* 444.91 (M+2, 100). *Anal*. Calcd. for ($C_{18}H_{11}BrN_4OS_2$): C, 48.76; H, 2.50; N, 12.64. Found: C, 48.81; H, 2.47; N, 12.69.

2.2.3.15. 2-((6-(4-Methoxyphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)methoxy) benzo[d]thiazole ***(19)***

Yellowish solid, yield: 1.05 g (53%), m.p. 238-241 ºC (from DMF-EtOH, 1:3); IR (ATR, cm^{-1}): 3097 (Ar-CH), 2978 (Aliph. CH), 1597 (C=N), 1169-1099 ($-OCH_3$); ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 3.74 (s, 3H, $-OCH_3$), 5.64 (s, 2H, $-CH_2$), Ar-H [8.05 (d, *J*=7.6 Hz, 1H), 7.90 (d, *J*=8.0 Hz, 1H), 7.74 (d, *J*=8.4 Hz, 2H), 7.48 (t, *J*=7.6, 8.0 Hz, 1H), 7.38 (m, 1H), 6.94 (d, *J*=8.8 Hz, 2H)], Imidazole-H [8.56 (s, 1H)]; ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm): 42.28 ($-CH_2$), 55.58 ($-OCH_3$), Ar-C [115.76 (CH), 121.64 (CH), 122.65 (CH), 125.43 (CH), 127.02 (C), 130.77 (CH), 135.58 (CH), 152.42 (C), 160.89 (C), 162.48 (C), 164.97 (C)], Imidazole-C [109.89 (CH), 145.64 (C)], Thiadiazole-C [144.78 (C), 163.34 (C)]; MS: *m/z* 396.48 (M+2, 51). *Anal*. Calcd. for ($C_{19}H_{14}N_4O_2S_2$): C, 57.85; H, 3.58; N, 14.20. Found: C, 57.89; H, 2.62; N, 14.23.

2.2.3.16. 2-((6-(4-Nitrophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)methoxy) benzo[d]thiazole ***(20)***

Brownish solid, yield: 1.37 g (67%), m.p. 246-248 ºC (from DMF-EtOH, 1:3); IR (ATR, cm^{-1}): 3152 (Ar-CH), 2928 (Aliph. CH), 1600 (C=N); ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 5.64 (s, 2H, $-CH_2$), Ar-H [8.25 (d, *J*=8.8 Hz, 2H), 8.07 (d, *J*=8.8 Hz, 2H), 8.04 (d, *J*=8.4 Hz, 1H), 7.90 (d, *J*=8.0 Hz, 1H), 7.48 (t, *J*=7.2, 7.2 Hz, 1H), 7.39 (t, *J*=7.6, 7.2 Hz, 1H)], Imidazole-H [8.94 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.38 ($-CH_2$), Ar-C [121.87 (CH), 122.60 (CH), 124.68 (CH), 125.41 (CH), 125.75 (CH), 127.07 (CH), 135.59 (C), 140.74 (C), 146.73 (C), 152.58 (C), 164.92 (C)], Imidazole-C [113.58 (CH), 146.59 (C)], Thiadiazole-C [143.48 (C), 163.85 (C)]; MS: *m/z* 410.19 (M41, 62). *Anal*. Calcd. for ($C_{18}H_{11}N_5O_3S_2$): C, 52.80; H, 2.71; N, 17.10. Found: C, 52.78; H, 2.74; N, 17.09.

2.2.3.17. 4-(2-((Benzo[d]thiazol-2-yloxy)methyl)imidazo[2,1-b][1,3,4]thiadiazol-6-yl)benzonitrile ***(21)***

White solid, yield:1.42 g (73%), m.p. 221-222 ºC (from DMF-EtOH, 1:2); IR (ATR, cm^{-1}): 3142 (Ar-CH), 29 89 (Aliph. CH), 2225 (CN), 1596 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.65 (s, 2H, -CH_2), Ar-H [7.98 (d, *J*=6.8 Hz, 2H), 7.84 (d, *J*=6.8 Hz, 2H), 7.71 (d, *J*=8.0 Hz, 1H), 7.46 (d, *J*=8.0 Hz, 1H), 7.38 (t, *J*=7.6, 7.6 Hz, 1H), 7.24 (t, *J*=7.6, 7.6 Hz, 1H)], Imidazole-H [8.88 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.28 (-CH_2), Ar-C [109.89 (C), 113.10 (CH), 121.76 (CH), 123.72 (CH), 124.30 (CH), 125.58 (CH), 127.37 (CH), 133.23 (CH), 136.48 (C), 138.65 (C), 169.64 (C)], Imidazole-C [112.02 (CH), 146.07 (C)], Thiadiazole-C [143.97 (C), 160.63 (C)], 119.43 (CN); MS: *m/z* 389.99 (M+1, 49). *Anal*. Calcd. for ($C_{19}H_{11}N_5OS_2$): C, 58.60; H, 2.85; N, 17.98. Found: C, 58.58; H, 2.88; N, 18.03.

2.2.3.18. 2-((6-(3,4-Dichlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)methoxy) benzo[d]thiazole ***(22)***

Light red solid, yield:1.49 g (69%), m.p. 230-232 ºC (from DMF-EtOH, 1:2); IR (ATR, cm^{-1}): 3123 (Ar-CH), 2977 (Aliph. CH), 1590 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.63 (s, 2H, -CH_2), Ar-H [8.00 (d, *J*=2.0 Hz, 2H), 7.76 (dd, *J*=2.0, 2.0 Hz, 1H), 7.69 (d, *J*=7.6 Hz, 1H), 7.61 (d, *J*=8.4 Hz, 1H), 7.45 (d, *J*=8.4 Hz, 1H), 7.38 (t, *J*=7.2, 8.0 Hz, 1H), 7.22 (t, *J*=8.0, 7.6 Hz, 1H)], Imidazole-H [8.77 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 22.25 (-CH_2), Ar-C [112.17 (CH), 121.77 (CH), 123.72 (CH), 124.29 (CH), 125.10 (CH), 126.58 (CH), 127.35 (CH), 129.94 (C), 131.37 (C), 131.99 (C), 134.86 (C), 136.45 (C), 169.64 (C)], Imidazole-C [111.99 (CH), 145.69 (C)], Thiadiazole-C [143.31 (C), 160.28 (C)]; MS: *m/z* 432.02 (M+1, 34). *Anal*. Calcd. for ($C_{18}H_{10}Cl_2N_4OS_2$): C, 49.89; H, 2.33; N, 12.93. Found: C, 49.93; H, 2.34; N, 12.89.

2.2.3.19. 2-((6-(4-Phenylphenyl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)methoxy) benzo[d]thiazole ***(23)***

Whitish solid, yield:1.26 g (57%), m.p. 258-261 ºC (from DMF-EtOH, 1:3); IR (ATR, cm^{-1}): 3127 (Ar-CH), 2979 (Aliph. CH), 1589 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.64 (s, 2H, -CH_2), Ar-H [7.90 (d, *J*=8.4 Hz, 2H), 7.70 (m, 4H), 7.41 (m, 4H), 7.24 (t, *J*=7.2, 7.6 Hz, 1H)], Imidazole-H [8.72 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.26 (-CH_2), Ar-C [112.03 (CH), 121.78 (CH), 123.72 (CH), 124.29 (CH), 125.66 (CH), 126.91 (CH), 127.37 (CH), 127.88 (CH), 129.38 (CH), 133.30 (C), 136.51 (C), 139.46 (C), 140.14 (C), 169.64 (C)], Imidazole-C [111.15 (CH), 146.53 (C)], Thiadiazole-C [145.34 (C), 159.61 (C)]; MS: *m/z* 440.58 (M^+, 67). *Anal*. Calcd. for ($C_{24}H_{16}N_4OS_2$): C, 65.43; H, 3.66; N, 12.72. Found: C, 65.46; H, 3.67; N, 12.70.

2.2.3.20. 2-((6-(Naphthalen-2-yl)imidazo[2,1-b][1,3,4]thiadiazol-2-yl)methoxy) benzo[d]thiazole ***(24)***

Brownish solid, yield: 1.14 g (55%), m.p. 221-223 ºC (from DMF-EtOH, 1:3); IR (ATR, cm^{-1}): 3054 (Ar-CH), 2983 (Aliph. CH), 1592 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.65 (s, 2H, -CH_2), Ar-H [8.36 (s, 1H), 7.91 (m, 4H), 7.71 (d, *J*=8.0 Hz, 1H), 7.46 (m, 4H), 7.39 (t, *J*=7.6, 8.0 Hz, 1H), 7.23 (t, *J*=7.6, 7.6 Hz, 1H)], Imidazole-H [8.78 (s, 1H)]; ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 42.28 (-CH_2), Ar-C [112.00 (CH), 121.90 (CH), 123.29 (CH), 123.82 (CH), 124.29 (CH), 126.29 (CH), 126.91 (CH), 127.37 (CH), 128.07 (CH), 128.39 (C), 128.65 (C), 131.63 (C), 132.86 (C), 136.49 (C), 169.65 (C)], Imidazole-C [111.52 (CH), 145.86 (C)], Thiadiazole-C [133.63 (C), 159.62 (C)]; MS: *m/z* 414.83 (M+1, 83). *Anal*. Calcd. for ($C_{22}H_{14}N_4OS_2$): C, 63.75; H, 3.40; N, 13.52. Found: C, 63.72; H, 3.39; N, 13.55.

2.3. Determination of *in vitro* Antileishmanial and Antibacterial Activities

2.3.1. Antileishmanial Activity

In this study, we set out to determine *in vitro* antileishmanial activities of the compounds against *Leishmania infantum* promastigote isolates using the microdilution Alamar blue assay method.

2.3.1.1. Preparation of Leishmania Infantum Promastigotes

Axenic standard MON-183 *Leishmania infantum* promastigote isolates were used in a study on antiparasitic activity. Standard isolates were grown in RPMI-1640 (Roswell Park Memorial Institute) (R8758 Sigma Aldrich, USA) medium which was added 10% Fetal Bovine Serum (FBS F4135 Sigma-Aldrich, USA), 1% Penicillin (P3032 Sigma-Aldrich, USA), and Streptomycin (S9137 Sigma-Aldrich, USA) (100,000 units of penicillin and 10 mg streptomycin) and passaged to ensure durability.

20 ml of standard promastigote isolate was taken from the medium, transferred into sterile falcon tubes, and centrifuged for 10 min at 1,000 g. The supernatant was removed, and the isolates were washed 3 times with sterile PBS (Phosphate Buffered Saline). Then, the parasite count was adjusted to approximately $2.5x10^7$ promastigotes/mL by performing a count in the RPMI-1640 medium and hemocytometer.

2.3.1.2. In vitro Antileishmanial Activity Test

The synthesized compounds were dissolved in Dimethyl Sulfoxide (DMSO)/H_2O (10%). Stock solutions of the compounds were prepared at concentration of 40 mg/mL by adding RPMI-1640 medium containing heat-inactivated 10% FBS and sterilized by filtering it through a sterile membrane filter at a porosity of 0.45-μm (Millipore, USA). Sterile 96-well microplates were used to test antileishmanial activity. The dilution ratios in the wells were set

to between 20,000 µg/mL and 625 µg/mL. The microdilution test with Alamar blue was performed as described above [31]. The 7th well was added only 100 µl stock solution as negative control, while the 8th well was added only 100 µL standard parasite isolate as positive control. After the microplates were incubated for 20 hours on a refrigerated incubator set to 27 °C, they were added to 20 µL of Alamar blue (Resazurin sodium salt R7017 Sigma Aldrich, USA) (prepared at 0.1 mg/ml and sterilized by filtration). The microplates were incubated for another 4 hours at 27 °C and visually assessed at the 24th, 48th, and 72nd hour. Also, a fresh preparation was made between the slide and cover glass by taking a 30 µL sample from each well in order to observe the vitality of promastigotes. The results of this were confirmed visually. Amphotericin B was used as the standard control drug. The test was repeated twice for each compound. The conversion of Alamar blue to its pink derivative was interpreted as continuing parasite growth in wells. No change in color was interpreted as that parasite growth had stopped.

2.3.2. Antibacterial Activity

In this study, *in vitro* antibacterial activities of the compounds against five selected bacteria isolates (Gram-negative) were determined using the microdilution Alamar blue assay.

2.3.2.1. Preparation of Standard Bacterial Isolates

The purpose was to assess minimal inhibitory concentrations (MIC) of the compounds tested for antibacterial activity against five different standard Gram-negative bacteria using the microdilution Alamar blue assay method (Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Susceptibility Testing). The standard *Escherichia coli* ATCC 25922, *Yersinia enterocolitica* ATCC 9610, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 700603, and *Proteus mirabilis* ATCC 25933 isolates

obtained from the American Type Culture Collection (ATCC) were used. The standard bacteria isolates were cultured using blood agar (Merck) and Eosin Methylene Blue Agar (EMB, Merck), vitalized by incubation at 37 °C overnight, and were checked for purity. The standard bacteria isolates were prepared at approximately $1.5x10^8$ cfu/mL bacteria count by suspending in sterile physiological saline solution according to the 0.5 McFarland turbidity standard.

2.3.2.2. In vitro Antibacterial Activity Test

The antibacterial activity test for the stock solutions of the compounds was performed using sterile 96-well microplates. Stock solutions of the compounds were prepared at a concentration of 40 mg/mL by adding Mueller Hinton Broth (MHB, Merck) medium, and were sterilized by filtering through a sterile membrane filter with a porosity of 0.45-µm (Millipore, USA). Firstly, 100 µl of MHB medium was added to each well. As described above, the dilution ratios of the wells were set to between 20,000 µg/mL and 625 µg/mL [32]. 100 µL of standard bacteria suspension set to 0.5 McFarland turbidity standard were then added to these wells. Negative and positive control wells were added and incubated at 37 °C. The microplates were incubated for 20 hours, 20 µL of Alamar blue was then added, and then everything incubated again for another 4 hours. The microplates were visually assessed for color change at the 24th and 48th hour. Samples were taken from each well using a loop for growth control in order to see whether there was growth or not in the blood agar medium. The antibacterial activity test was repeated twice for each compound; Amikacin was used as control drug.

2.4. Computational Study

Molecular docking studies were performed by using Autodock Vina [27] and Discover Studio Visualizer 4.5 [33]. Optimized structures of ligands (**4a**, **4b**, **7**, and **13)** were calculated using the 6-31G(d,p) base set DFT (B3LYP) theory on Gaussian 09.

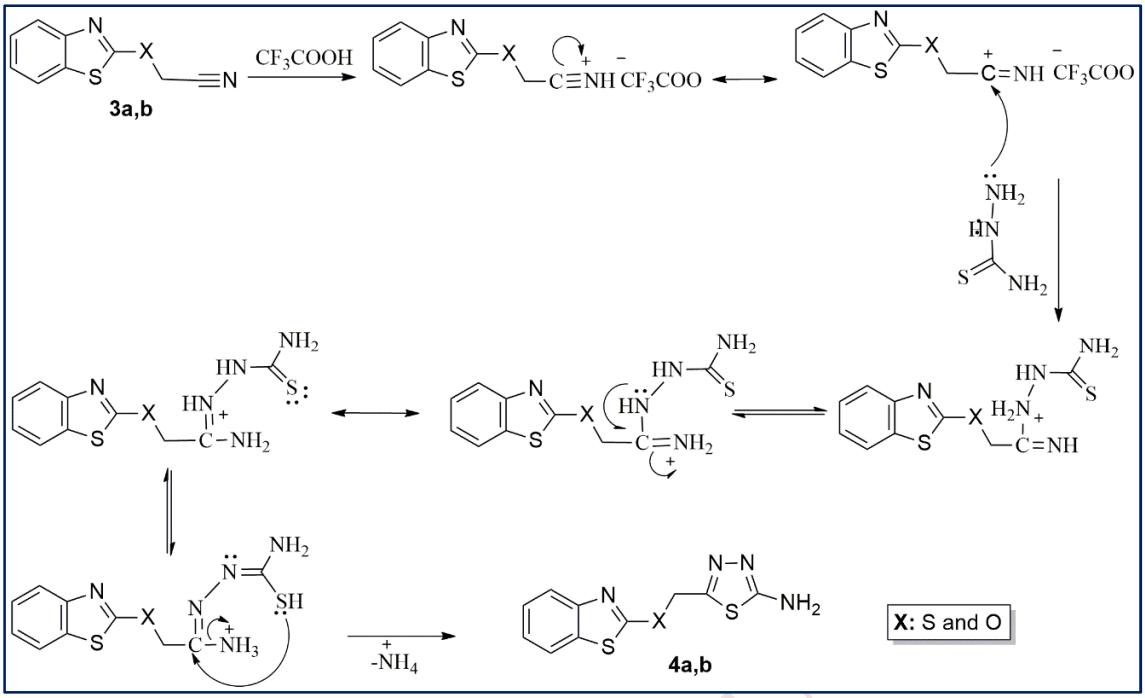
3. Results and discussion

3.1. Chemistry

In this study, we firstly synthesized benzo[*d*]thiazol-2-ylthio/oxy)acetonitrile compounds **(3a,b)**. The nitrile derivatives were obtained from the reaction of benzo[*d*]thiazole-2-thiol/ol compounds **(1a,b)** with chloroacetonitrile in high yields (82% and 87%).

In the second part of the study, we synthesized 2-amino-1,3,4-thiadiazole derivatives **(4a,b)** from the reaction of compounds **3a,b** with thiosemicarbazide in trifluoroacetic acid (TFA) in high yields (83% and 84%). Compounds **3a,b** and **4a,b,** which served as the starting compounds, were obtained as specified in the literature [24,34].

In this reaction, an addition occurs as a result of the thiosemicarbazide's nucleophilic attack on the positively-charged iminium carbon, which forms under the catalytic effect of TFA. It is believed that the elimination of the ammonia ion and the sulfur's nucleophilic attack on the carbon atom results in heterocyclization, which leads to the formation of 2-amino-1,3,4-thiadiazole derivatives **(4a,b)** [25,35]. The formation mechanism of these compounds can be seen in Scheme 2.



Scheme 2. The mechanism of the formation of 2-amino-1,3,4-thiadiazole derivatives **(4a,b)**.

Sharp absorption bands belonging to the -C≡N group observed at 2249 and 2263 cm^{-1} in the IR spectra of compounds **3a,b** disappeared. Symmetric and asymmetric absorption bands belonging to the -NH_2 group emerged in the 3271-3089 cm^{-1} range, which is the most important evidence for the formation of compounds **4a,b**.

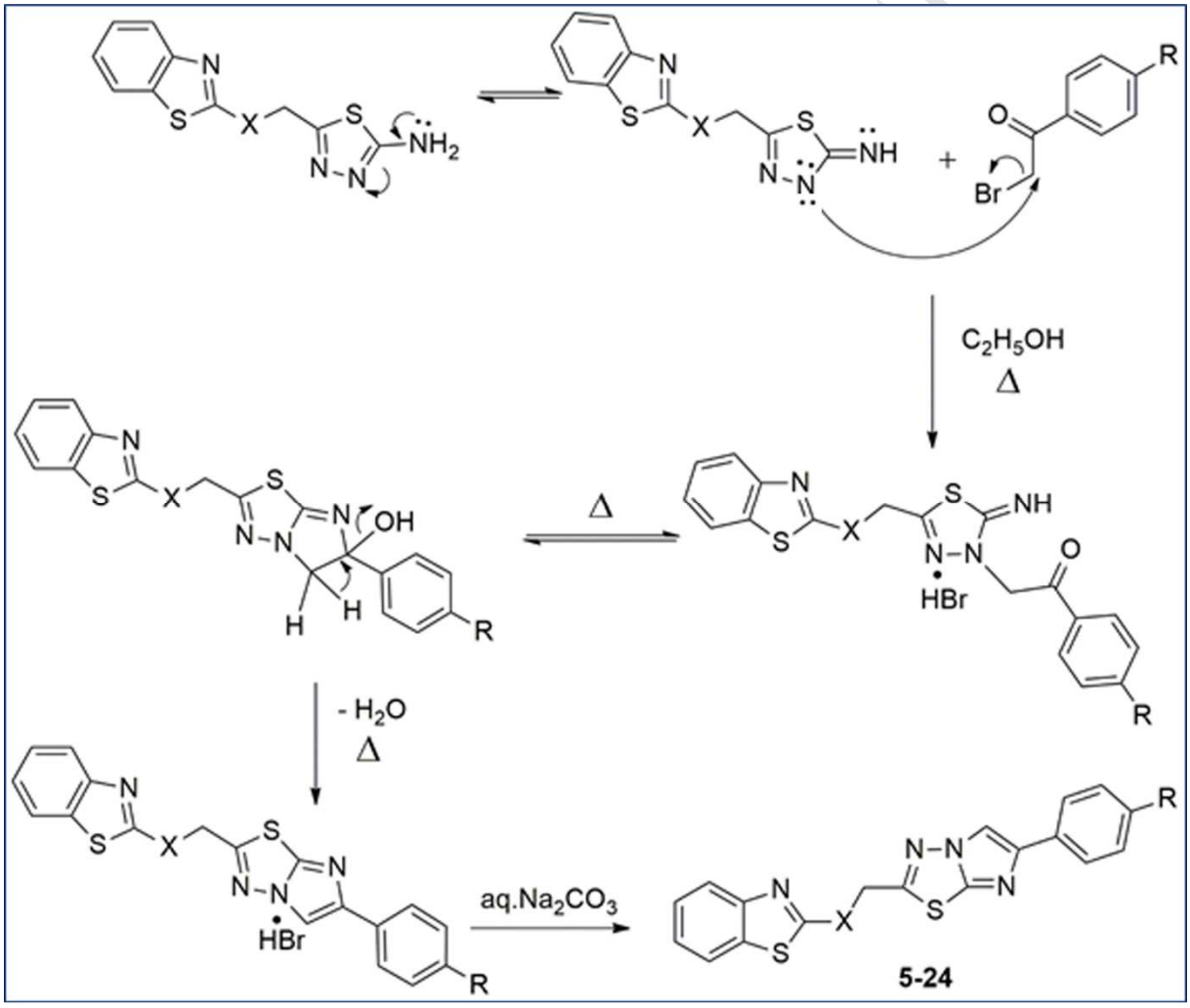
Structures of compounds **4a,b** were confirmed using ^1H NMR spectroscopy. The -NH_2 group proton signals of these compounds bonded to 1,3,4-thiadiazole ring from C_2 position in ^1H NMR spectra were observed as a singlet corresponding to 2 protons in the 7.14-7.24 ppm range.

Proton peaks belonging to the -NH_2 group of these compounds **(4a,b)** disappeared as a result of proton-deuterium exchange performed with D_2O.

Structures of compounds **4a,b** were confirmed by ^{13}C NMR spectrum. C_2 carbon signals belonging to the thiadiazole ring in these compounds appeared at 154.48 and 152.14 ppm. C_5 carbon signals of the same ring were recorded as 165.49 and 169.24 ppm. Other spectral data belonging to the carbon skeleton of the molecule fully support the suggested structures. The

^{13}C NMR spectra of these compounds were observed to be highly compatible with this type of compounds in the literature [3,25,35].

In the third part of the study, we synthesized the imidazo[2,1-*b*][1,3,4]thiadiazole derivatives **(5-24)**, the target compounds, from reactions of compounds **4a,b** with phenacyl bromide derivatives (in yields of 53% to 73%). Scheme 3 shows the formation mechanism of the target compounds.



Scheme 3. Formation mechanism of target compounds **(5-24)**.

In IR spectra of compounds **4a,b**, -NH_2 group symmetric and asymmetric absorption bands found in the initial compounds and observed in the 3271-3089 cm^{-1} range disappeared, which is the most important evidence for the formation of the target compounds.

Also, -NH_2 group proton signals observed at 7.14 and 7.24 ppm in the ^{1}H NMR of the starting compounds **(4a,b)** disappeared in these compounds and instead observed as a singlet corresponding to 1 proton in the 8.94-8.51 ppm range of the C_5-H signals in the imidazo[2,1-*b*][1,3,4]thiadiazole derivatives **(5-24)**, this is the most important evidence for the formation of these compounds. This is consistent with the literature [35]. Other ^{1}H NMR data of these compounds is given in the experimental part of this study in detail.

Signals that appeared in the 113.58-109.89 ppm range and the 152.80-145.25 ppm range in ^{13}C NMR spectrum of these compounds is important evidence for ring cyclization. These signals correspond to C_5 and C_6 carbons of imidazo[2,1-*b*][1,3,4]thiadiazole derivatives **(5-24)** or the target compounds. Other ^{13}C NMR spectrum data of these compounds is given in the experimental part in detail.

The FT-IR, ^{1}H NMR, ^{13}C NMR, elemental analysis, and mass spectroscopy of all compounds synthesized in the study can be found in the experimental section. All of the spectra are given in the Supplementary Material Section in detail.

3.2. *In vitro* Antileishmanial Activity Studies

Figure 1 shows the images of 21 compounds and Table 1 shows the minimum inhibitory concentration (MIC) values obtained as a result of the assessment. Positive and negative control wells were observed to work properly.

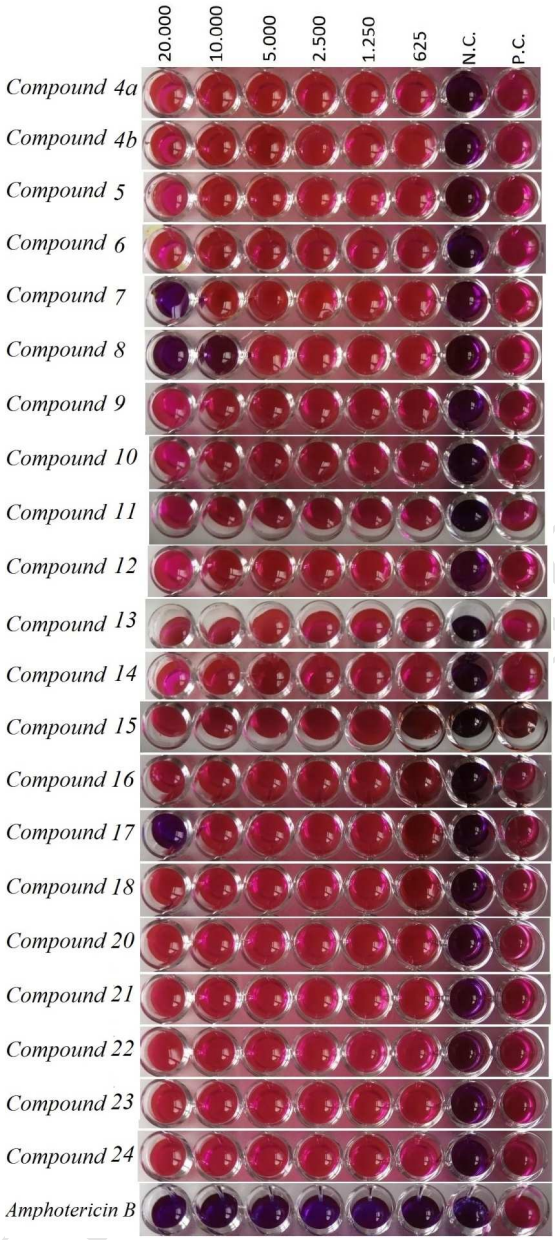


Figure 1. The antileishmanial activity results against axenic standard MON-183 *Leishmania infantum* promastigotes; compounds **4a,b** and **5-24**, Control drug Amphotericin B.
Dilution concentrations 20,000 µg/ml - 625 µg/ml.
N.C.: Negative Control; P.C.: Positive Control.

Table 1. Minimum inhibitory concentration (MIC) values of the compounds against *Leishmania infantum* promastigotes.

Compounds	MIC values (µg/ml)
4a	>20 000
4b	>20 000
5	>20 000
6	>20 000
7	20 000
8	10 000
9	>20 000
10	>20 000
11	>20 000
12	>20 000
13	>20 000
14	>20 000
15	>20 000
16	>20 000
17	20 000
18	>20 000
20	>20 000
21	>20 000
22	>20 000
23	>20 000
24	>20 000
Amphotericin B	<625

The antileishmanial activity study showed that compound **8** (MIC 10 000 µg/mL) was the most effective compound. Also, compounds **7** and **17** were found to be effective at the highest concentration studied (MIC 20 000 µg/mL). Other compounds had no antileishmanial activity at the studied concentrations.

Amphotericin B, or the standard drug, was found to be effective at a concentration <625 µg/ml.

3.3. *In vitro* Antibacterial Activity Studies

While some of the synthesized compounds were observed to have antibacterial activity against standard bacteria isolates at different concentrations, some compounds were ineffective at the studied concentrations.

Bacterial activity images of some compounds can be seen in Figures 2 and 3. Minimum inhibitory concentration (MIC) values of the compounds can be seen in Table 2.

As it was found that the synthesized compounds were effective against five different standard bacteria isolates at varying degrees. The compounds were most effective against *Escherichia coli*. All compounds except for compound **17** were found to be effective at different concentrations (MIC=625-10 000 µg/mL). Compounds **4b** and **7** were found to be the most effective compounds against *Escherichia coli* (MIC=625 µg/mL). The second bacteria on which the compounds were effective was *Yersinia enterocolitica* (MIC= 1 250-20 000 µg/mL). Compound **4b** was once again observed to be the most effective compound against *Yersinia enterocolitica* (MIC=1 250 µg/mL) and compounds **4b** and **24** were found to be effective against *Salmonella typhimurium* (MIC= 2 500 and 5 000). Except for compound **24** (MIC=10 000 µg/mL), none of the compounds was effective against *Klebsiella pneumoniae*. None of the compounds was effective against *Proteus mirabilis* at the studied concentrations (MIC >20 000).

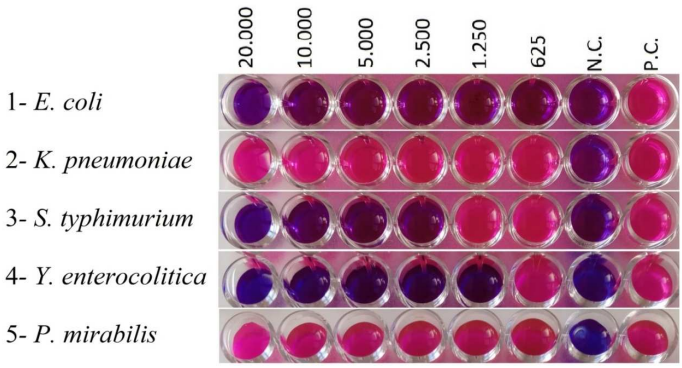


Figure 2. Antibacterial activity results of compound **4b**; Dilution concentrations 20 000 µg/ml-625 µg/ml. N.C.: Negative Control; P.C.: Positive Control.

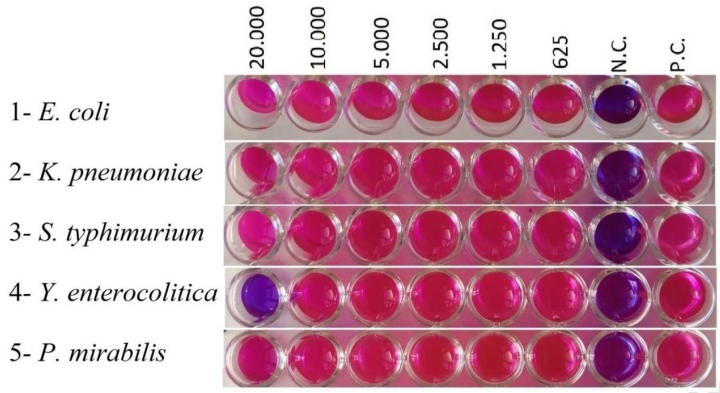


Figure 3. Antibacterial activity results of compound **17**; Dilution concentrations 20,000 µg/ml-625 µg/ml. N.C.: Negative Control; P.C.: Positive Control.

Table 2. Minimum inhibitory concentration (MIC) values against bacteria of all compounds.

Compounds	*E. coli*	*K. pneumoniae*	*S. typhimurium*	*Y. enterocolitica*	*P. mirabilis*
4a	1 250	>20 000	10 000	2 500	>20 000
4b	625	>20 000	2 500	1 250	>20 000
5	5 000	>20 000	>20 000	>20 000	>20 000
6	2 500	>20 000	>20 000	>20 000	>20 000
7	625	>20 000	10 000	5 000	>20 000
8	2 500	>20 000	10 000	5 000	>20 000
9	5 000	>20 000	>20 000	>20 000	>20 000
10	5 000	>20 000	>20 000	>20 000	>20 000
11	10 000	>20 000	10 000	2 500	>20 000
12	10 000	>20 000	10 000	10 000	>20 000
13	>20 000	>20 000	>20 000	>20 000	>20 000
14	10 000	>20 000	>20 000	>20 000	>20 000
15	5 000	>20 000	10 000	20 000	>20 000
16	10 000	>20 000	10 000	10 000	>20 000
17	10 000	>20 000	>20 000	20 000	>20 000
18	10 000	>20 000	>20 000	20 000	>20 000
20	10 000	>20 000	>20 000	>20 000	>20 000
21	10 000	>20 000	>20 000	>20 000	>20 000
22	10 000	>20 000	>20 000	>20 000	>20 000
23	10 000	>20 000	>20 000	>20 000	>20 000
24	5 000	10 000	5 000	10 000	>20 000

3.4. Molecular Docking Studies

Molecular docking studies of compounds **4a**, **4b**, **7**, and **13** were performed using Autodock Vina [27] software. Receptor-ligand interactions were demonstrated with Discover Studio Visualizer 2017 [33]. Molecular docking studies were performed as X-ray crystal structure belonging to the *Escherichia coli* protein structure (PDB ID: 2eg7). Before proceeding to the calculations, the crystal structure of 2eg7 and all ligand compounds were prepared using protein and ligand preparation wizards in the PyRx software [36]. Prior to the docking process, firstly water and heteroatoms other than natural ligand (***co-ligand***) were removed from and hydrogen atoms, and Gasteiger charges were then added to the 2eg7 protein structure.

The optimized structures of all ligands were obtained using the DFT(B3LYP) [28,29] theory with the 6-31G(d) basis set on Gaussiam 09.

Molecular docking studies were performed to see possible bonding modes of the synthesized compounds. The co-ligand and complex 2eg7 X-ray crystal structure was used in calculations. The *in vitro* antibacterial activity studies (Table 2) showed that the most effective compounds against the *E. coli* disease were compounds **4a**, **4b**, and **7**, while compound **13** was found to be ineffective. Docking studies were performed for these three ligands as well as ligand **13**. Table 3 shows the docking scores of compounds **4a**, **4b**, **7**, and **13** and the co-ligand, interactions between the receptors and ligands, and HOMO energy levels of the ligands (Figure 4) comparatively. Figures 5-9 show the docking operations to the active binding sites of the target protein.

Table 3. Molecular modeling data for **co-ligand**, **4a, 4b, 7** and **13** during docking in the active site of enzyme 2eg7.

Compound no.	Affinity (kcal/mol)	No. of hydrogen bonds	Residue	Distance D-H…A (Å)	Functional groups	HOMO (eV)
Co-ligand	-6.7	6	HIS177	2.07	C=O	-7.33
			HIS139	2.32	C=O	
			ASP250	3.38	N	
			ASN44	2.16	C=O	
			ARG20	1.90	C-O	
			LEU222	1.96	C=O	
			LEU222	3.39	N	
4a	-6.2	3	ASN44	2.83	N	-6.04
			LEU222	2.24	NH	
			ASP250	2.25	NH	
4b	-6.3	4	ASN44	2.97	O	-6.16
			ASN44	2.26	N	
			LEU222	2.07	N	
			LEU222	2.53	NN	
			ASP250	2.36	NH	
7	-5.6	2	ASN44	2.41	N	-5.90
			ASN44	2.31	N	
13	-1.9	-	-	-	-	-5.58

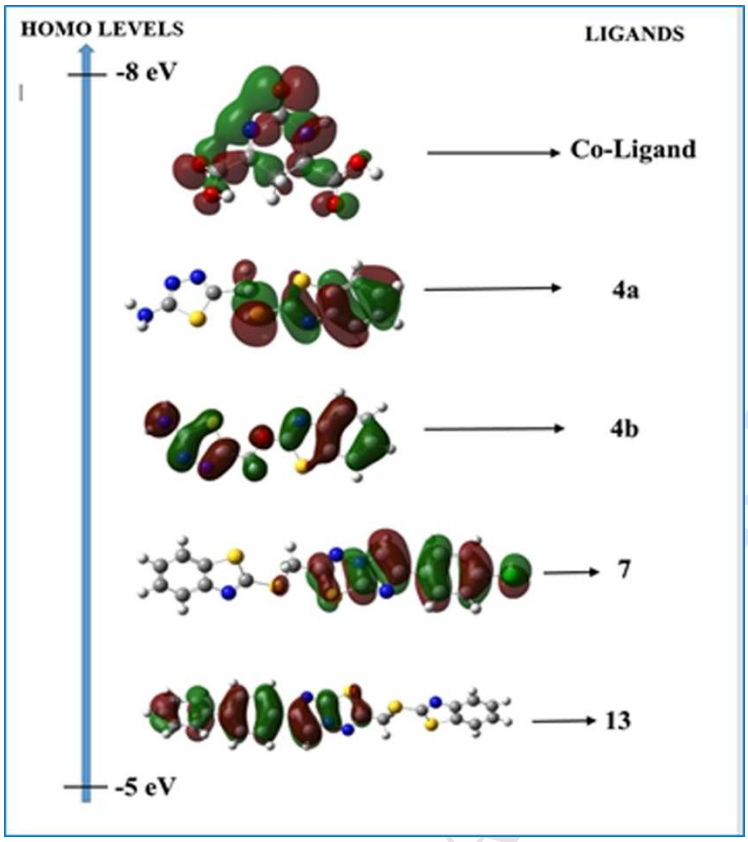


Figure 4. Representation of HOMO orbitals of **co-ligand**, **4a**, **4b**, **7**, and **17**.

It was seen that the ligand bonded to active bonding site of receptors with weak, non-covalent interactions and, more specifically, with hydrogen bonding and alkyl interactions. As can be seen in Figure 5, O atom of the receptor and the co-ligand formed hydrogen bonds in lengths of 2.09, 2.37, 2.26, 2.64, 1.98, 2.06 Å with HIS177, THR366, HIS139, HIS254, ASN44, ARG20, and LEU222 residues of the receptor, respectively. The N atom of ligand **4b** formed hydrogen bonds in lengths of 2.78, 2.80, 2.09, and 2.56 Å with HIS118, ASN44, LEU222, and ASP250 of the receptor, respectively (Figure 7). Similarly, interactions of ligands **4a**, **7**, and **13** with the receptor are shown in Table 3. These three ligands were observed to interact similarly with the co-ligand according to their calculated binding affinity, while ligand **13** was observed to not form a classical hydrogen bond other than electrostatic and hydrophobic interactions. HOMO and LUMO orbitals are used to explain ligand-receptor interactions. The molecular mechanism of the ligand binding site was explained as follows at the quantum

level: HOMO orbitals on the nucleophilic molecule (drug) interact with LUMO orbitals on the electrophilic agent (the active site of the enzyme) [37]. It was previously reported that the ligand with high HOMO energy had better interactions with the receptor and matched better with experimental activity studies [35]. It is seen that ligands with high affinity values according to Table 3 had high HOMO energy. In conclusion, it is possible to say that ligands **4a**, **4b**, and **7** could be candidate inhibitor molecules for the target structure 2eg7. The findings obtained in this study leads to the idea that these compounds could be new potential inhibitors with biological activity for the 2eg7 protein structure, and that they could also be used in *in vitro* studies.

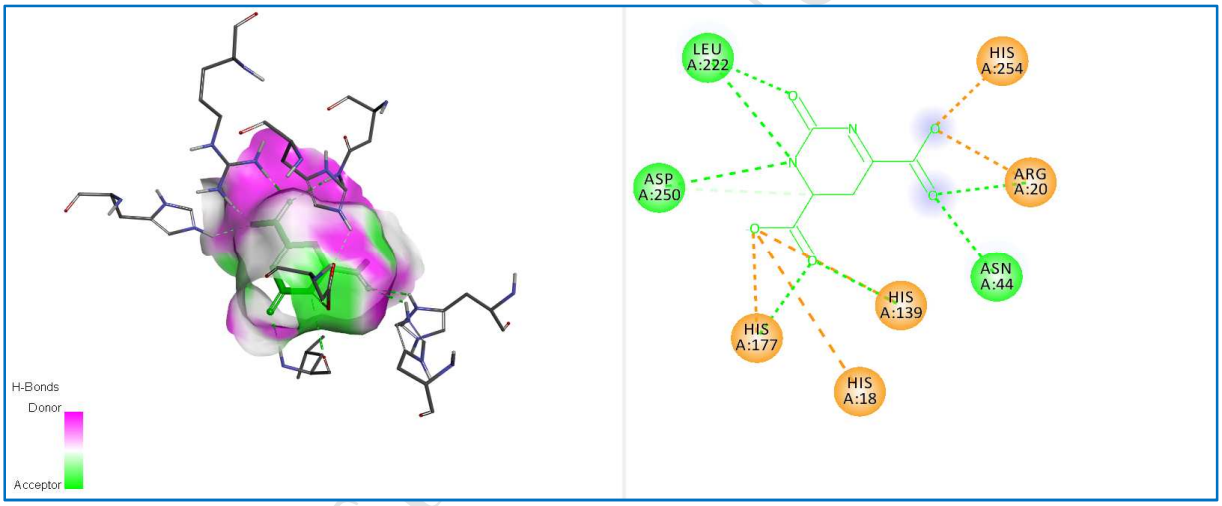


Figure 5. The 3D and 2D presentation of docking results of co-ligand in the active region of the 2eg7 protein.

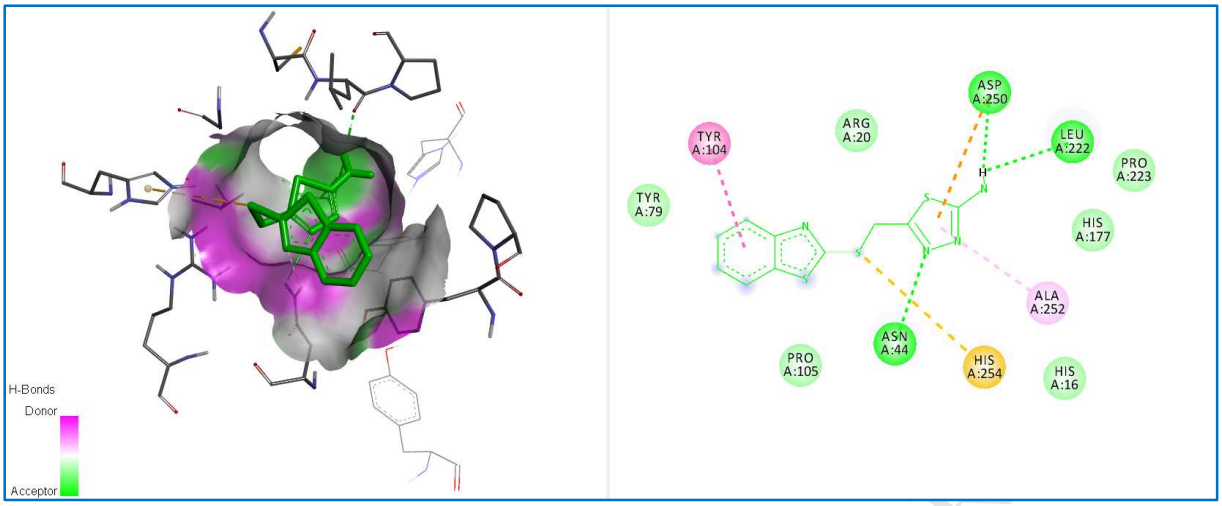


Figure 6. The 3D and 2D presentation of docking results of ligand **4a** in the active region of the 2eg7 protein.

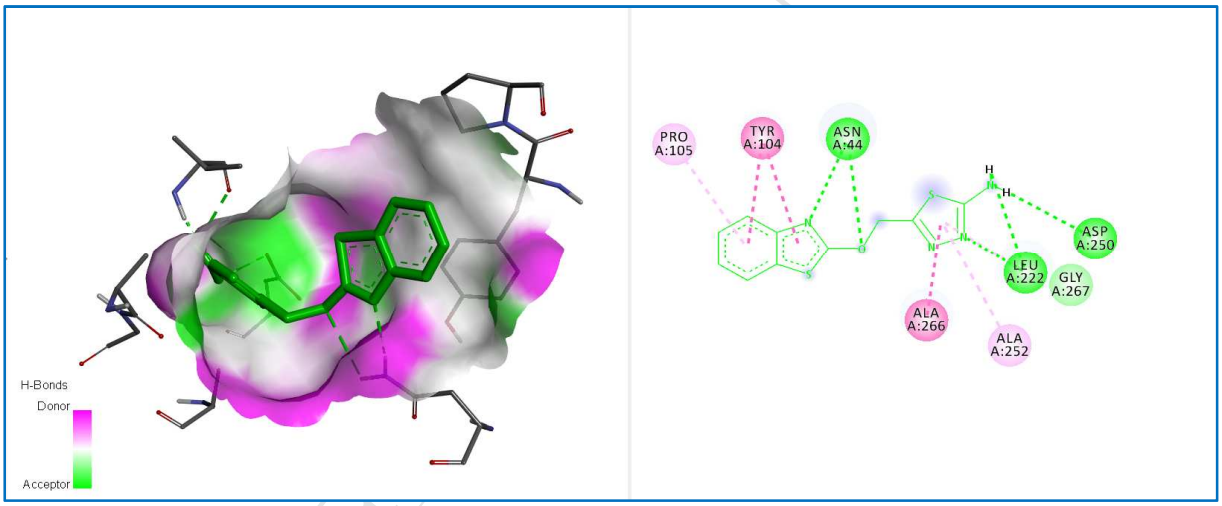


Figure 7. The 3D and 2D presentation of docking results of ligand **4b** in the active region of the 2eg7 protein.

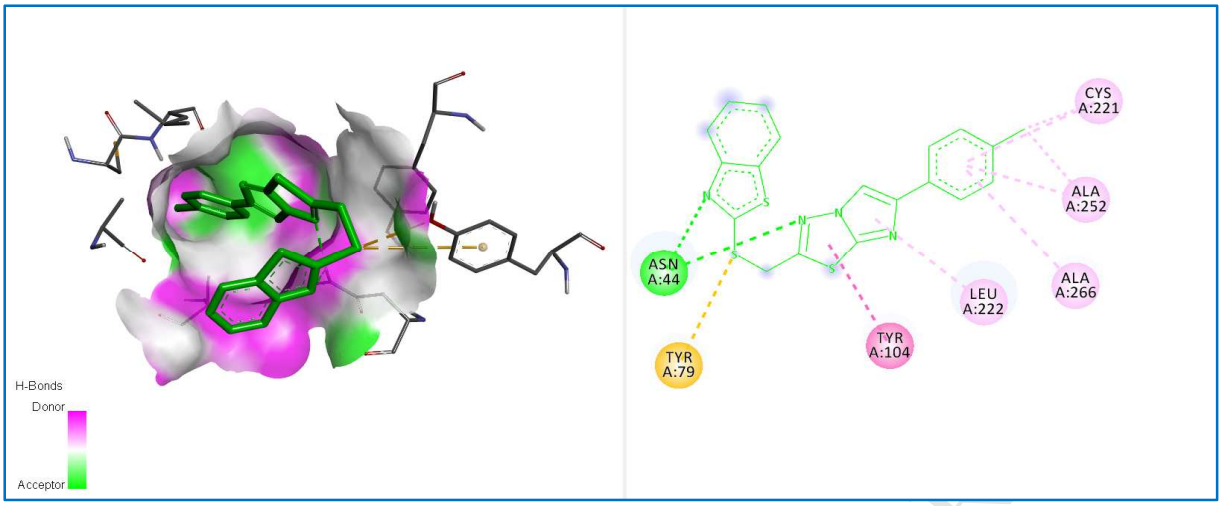


Figure 8. The 3D and 2D presentation of docking results of ligand **7** in the active region of the 2eg7 protein.

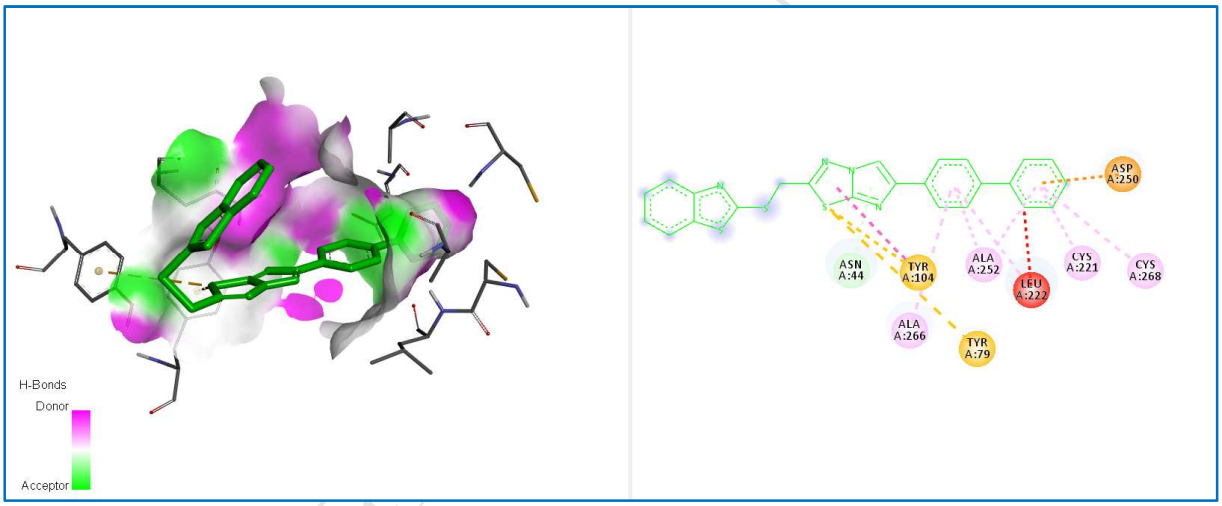


Figure 9. The 3D and 2D presentation of docking results of ligand **13** in the active region of the 2eg7 protein.

4. Conclusions

In this study, we synthesized target compounds containing both benzothiazole and imidazo[2,1-*b*][1,3,4]thiadiazole derivatives using simple reaction methods. All of the compounds synthesized were characterized using various analysis methods.

All of the compounds were tested for *in vitro* antileishmanial and antibacterial activity. According to the test results, compound **8** was found to be effective as an antileishmanial substance (MIC=10 000 µg/ml). It was also concluded that this compound could be further studied as a potential drug ingredient. This study moreover revealed that compounds **4a**, **4b**, **7**, **11**, and **24** were effective (MIC=625>5 000 µg/ml). Control studies with experimental *in vivo* animal models are required for the synthesized compounds to be used as drug ingredients.

After calculating the stable structures of the selected compounds (**4a**, **4b**, and **13**), the docking simulation process was used to find possible bonding models as well as to confirm the molecule. The docking results obtained in the study showed that compounds **4a**, **4b**, and **7** could be new potential inhibitor compounds for the 2eg7 protein structure.

Acknowledgments

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Highlights

- Novel imidazo[2,1-*b*][1,3,4]thiadiazole derivatives were synthesized and characterized
- *In vitro* antileishmanial and antibacterial tests were applied to the compounds
- Theoretical calculations were performed to support the experimental results
- The HOMO energies of the compounds **4a**, **4b**, **7** and **13** were calculated
- Molecular docking study was performed for the compounds **4a**, **4b**, **7** and **13**