



Designs, synthesis, structural elucidation and antimicrobial evaluation of various derivatives of 2-mercaptobenzimidazole as possible antimicrobial agents

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ABSTRACT

Objective: Derivatives of 2-Mercaptobenzimidazole (2-MBI) are very significant heterocyclic compounds. 2-MBI and its derivatives have been showing different biological activities, the most significant activity exhibiting the antimicrobial activity. The aim of the project includes the synthesis of new derivatives of 2-MBI. **Methods:** Formation of 2-(Phenethylthio)-1H-benzo[d]imidazole (ZR-1) by alkylation, thioester S-(1H-benzo[d]imidazol-2-yl) O-ethyl carbonothioate (ZR-2), S-(1H-benzo[d]imidazol-2-yl) O-isobutyl carbonothioate (ZR-4), and S-(1H-benzo[d]imidazol-2-yl) O-methyl carbonothioate (ZR-8), the reaction involves thio acetic derivative 2-((1H-benzo[d]imidazol-2-yl) thio) acetic acid (ZR-3) with oxalyl chloride to generate acyl derivatives to this added cyclic amines to yield compounds, amid derivatives 2-((1H-benzo[d]imidazol-2-yl) thio)-1-(2, 6-dimethylpiperidin-1-yl) ethan-1-one (ZR-5), 2-((1H-benzo[d]imidazol-2-yl) thio)-1-(pyrrolidin-1-yl) ethan-1-one (ZR-6), and 2-((1H-benzo[d]imidazol-2-yl) thio)-1-(4-methylpiperazin-1-yl) ethan-1-one (ZR-7). **Results:** Structure of newly synthesized compounds have been verified through FT-IR, DSC, Elemental analysis, 1H-NMR, 13C-NMR, and Molecular docking, it indicates one of these compounds (ZR-5) has great affinity (-8.70) than our standard (trimethoprim -7.91 Kcal/mol. into DHFR). An antimicrobial activity was evaluated by agar diffusion methods and broth dilution test against *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus subtilis* (ATCC 6633) and *Candida albicans* (ATCC 10231). The Minimum Inhibitory Concentrations (MIC) and the Minimum Bactericidal Concentration (MBC) were determined and compared with ciprofloxacin (85.51%), trimethoprim and fluconazole (99.9%) as standard positive control drugs. **Conclusion:** Compound (ZR-8) showed excellent antifungal activity and good antibacterial activity compared to ciprofloxacin and trimethoprim. The results promoted the interest to do more structural modifications to enhance the antimicrobial activity and their antimicrobial selectivity.

Keywords: 2-mercaptobenzimidazole, Alkylation, Amidation, Acyl formation

INTRODUCTION

Benzimidazole is an important pharmacophore and a privileged structure in medicinal chemistry, is a heterocyclic aromatic organic compound (Figure 1) [1]. Benzimidazole is bicyclic in nature which consists of the fusion of benzene and imidazole [2]. Derivatives of benzimidazole were synthesized with the substitution of Fluorine, Propylene, tetrahydroquinoline which lead to compounds with increased stability, bioavailability and significant biological activity [3].

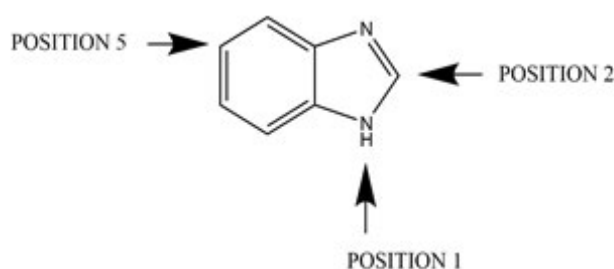


Figure 1 Benzimidazole

Benzimidazole containing compounds have different medical and biological activities, such as antitumor, antibacterial, antifungal, antiviral, anticonvulsant, antidepressant, analgesic, anti-inflammatory, antidiabetic properties [4-11]. Almost all benzimidazole derivatives with their two ring systems afford different functional substituents and this leads to essential modification of the metabolic and pharmacokinetic properties physico-chemical, of these drugs. In the past few decades, benzimidazole and its derivatives have received much attention because of their chemotherapeutic values, Antioxidant Activity Oxygen-derived, Antimicrobial and Antibacterial Activity Benzimidazole shows their antibacterial activity by inhibiting the bacterial nucleic acid and proteins synthesis [12]. Benzimidazole and imidazole drugs have wide range in remedying different dispositions in clinical medicine [13]. Imidazole nucleus forms the main structure of some well-known components of human body, that is, the amino acid histidine, Vit-B12, a component of DNA base structure and purines, histamine, and biotin [14]. There are drugs of Imidazole have a broader scope in treatment of different dispositions in clinical medicine [14]. It is also present in the structure of many natural or synthetic drugs molecules. 2-mercaptobenzimidazole derivatives, one of the most important derivatives of benzimidazole exhibited a wide variety of interesting biological activities such as antimicrobial, antihistamine, neutropic, and analgesic activities [15-18].

2-Mercaptobenzimidazole derived from benzimidazole with thiol group in the 2-position (Figure 2). It possesses other chemical names such as, o-phenylen thiourea, benzimidazol-2-thion with formula of $C_7H_6N_2S$ [19]. Some characteristic of 2-mercaptobenzimidazole are containing of thioamide group ($-N-C=S$), therefore it is considered one of thioamide compounds for its ability to react under special condition to give derivatives having substituent at either nitrogen or sulfur atoms, 2-mercaptobenzimidazole possess the form dimer, because it has $(C=S)$ group, this preferable product is the dimer, it is known to exist in two tautomerism forms, the thiol and thion form as represented below [20, 21].

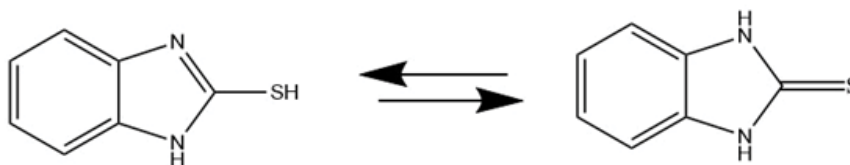


Figure 2 2-mercaptobenzimidazole

In reviewing most of antimicrobial drugs or possible investigated antimicrobial agent we envision the addition of groups that changes the nature of the thiol group in 2-mercapto benzimidazole. Thiol conversion in to various derivatives reflex new compounds with different interaction with the site of action. Cyclic amine may exist in different conformation that generates different activity. The hydrocarbons (ethyl, methyl, isobutyl) represent various in lipophilicity. $COOH$ (R) hydrophilic in nature and could be converted in to various derivatives. Unique changes in groups are expected to generate effective antibacterial and antifungal agent.

MATERIAL AND METHODS

Chemicals

The following chemicals was used: 2-mercaptobenzimidazole (98%), Piperidine 99%, 2,6 Dimethyl piperidine, Pyr-

rolidine, Methyl chloroformate, Ethyl chloroformate, Isobutyl chloroformate, Oxalyl chloride 99%, 2-Chloroethanol 99%, Chloroacetic acid 99%, 2-Bromoethyl benzene 98% all of them (Sigma Aldrich USA), Magnesium sulfate anhydrous (Lonover, UK), Potassium bromide (KBr) (Scharlau, Spain), Potassium carbonate anhydrous (K_2CO_3) (Gainland Chemical Company (GCC), UK), Potassium hydroxide (KOH) (Lonover, UK), N, N-Dimethylformamide (DMF) (AZ chem, Canada), Absolute ethanol 99.9% (Super chem, India), Dichloromethane 99.8% (Medex, UK), Acetone 99% (Scharlau, Spain). Chloroform extra pure (TEDIA, USA), Distilled water (Ultra, Joran).

Instrumentation

Analytical balance with a precision 0.01 mg (Phoenix instrument, USA), hot plate with magnetic stirrer (IKAMAG, India), Rotary evaporator (Roker 600, Germany), Buchner Funnel Pump (Vacuubrand, Germany). Melting point was determined on Melting Point Apparatus (GallenKamp, Germany). The structures of the synthesized compounds were confirmed by Differential Scanning Calorimetry (DSC) (Mettler Toledo, USA), Nuclear Magnetic Resonance (NMR) 300 MHZ (Varian 300 MHZ, Bruker, USA), elemental analysis was performed for C, H, N using Euro EA elemental analyzer, the results obtained had a maximum deviation of (± 0.4) from the theoretical value, which is considered within the acceptable variation range in results ($\pm 4\%$) (Euro Vector, Italy). This variation range is set according to the accuracy of Euro EA elemental analyzer device. FT-IR (Perkin Elmer 7600 Germany), Infra-Red (IR). DSC thermogram measurement was carried out by using the DSC 1 stare system v.11.ox. ChemBioDraw (Massachusetts, U.S.A) was used in the drawing of our schemes.

Docking

The Dihydrofolate Reductase (DHFR) protein target was selected from RCSB databank. The DHFR complex with inhibitor with PDB id 3GHW was downloaded for study. The NADPH is an important cofactor in DHFR activity. Thus the enzyme complex with NADPH was extracted. The complex was then used for minimizing the structure. The GROMACS, Linux based software was used for DHFR and NADPH complex minimization. The DHFR and NADPH complex was used as a docking target enzyme. The ligand structure was constructed using ChemDraw 2D. Then it was used to get three-dimensional structures of ligand using Chem3D. Also the ligand was energy minimized using MM2 force field present in Chem3D. Then, ligands and enzyme were prepared for docking using AutoDock Tools. The grid for docking was prepared using the inhibitor site and used it as a template. The Lamarck GA was used to perform the docking. The docking was performed for 10 conformations and done used using AutoDock 4.2. The results were then analyzed using PyMOL and Chimera visualization software.

Selected Test Microorganisms

Staphylococcus aureus (*S. aureus* ATCC 6538), *Bacillus subtilis* (*B. Subtilis* ATCC 6633), *Pseudomonas aeruginosa* (*P. aeruginosa* ATCC 9027), *Escherichia coli* (*E. coli* ATCC 8739), *Candida albicans* (*C. albicans* ATCC 10231), all these pure cultures of bacterial and fungal strains were obtained from Dar Al-Dawa (Na'ur, Jordan).

Culture Media

Muller Hinton Broth (MHB) (Mastgrp Ltd, UK), Mueller Hinton Agar (MHA) (Mastgrp Ltd, UK)/ (Himedia, India), Sabourauds Dextrose Broth (SDB) (Himedia, India), Sabourauds Dextrose Agar (SDA) (Mastgrp Ltd, UK).

Evaluation of Antimicrobial Activity

The antimicrobial activity of the newly synthesized compounds of different derivatives of 2-mercaptobenzimidazole (ZR1-ZR8) were evaluated by agar diffusion method against Gram-positive bacteria, Gram-negative bacteria and fungi, using *S. aureus* (ATCC 6538), *E. coli* (ATCC 8739), *P. aeruginosa* (ATCC 9027), *B. subtilis* (ATCC 6633), and *C. albicans* (ATCC 10231). Mueller Hinton agar or Sabourauds dextrose agar was used in a petri dish and overwhelm its surface with 0.1 ml (1×10^6 CFU/ml) inoculum of the required microorganism, Mueller Hinton agar is used for bacteria, while Sabourauds dextrose agar for fungi [22]. Then wells were cut and filled with 0.2 ml of the synthesized compounds (ZR1-ZR8) in concentrations of 500 μ g/ml. The plates were left at room temperature for 30 min, and then the plates were incubated at 37°C for 24 h for bacteria, and at 25°C for 48 h for fungi. The zone of inhibition was determined by measuring the diameter of the zone formed around each wells and the results were compared with a positive control (100 μ g/ml ciprofloxacin and trimethoprim 200 μ g/ml dissolved in DMSO for bacteria, 500 μ g/ml miconazole dissolved in DMSO for fungi), and negative control (DMSO alone) was included also [23].

The Minimum Inhibitory Concentration (MIC) against both bacteria and fungi and the Minimum Bactericidal or Fungicidal Concentration (MBC/MFC) was determined by microplate broth dilution technique. Briefly, MIC, MBC

or MFC were determined by the broth twofold micro dilution method in Mueller Hinton broth or Sabourauds dextrose broth, according to a modification of the procedure reported by NCCLS, 2012 [24]. Compounds (ZR-1 to ZR-8) were dissolved in DMSO (1 ml) to obtain concentration of 500 $\mu\text{g/ml}$ as stock solutions. The serial dilution method was used to obtain (250 to 0.488) $\mu\text{g/ml}$ concentrations. Mueller-Hinton broth was used as medium for bacterial growth, 100 μl of each concentration were distributed in 96-well plates with the exception of those wells acting as growth control (contain microorganisms and culture media) and positive control (contain microorganisms and standard control antibiotic), 20 μl of the standard microorganism's inoculum (equivalent to 1×10^6 CFU) were added to each well except the negative control well, the microplates were covered and sealed with parafilm and incubated at 37°C for 18-24 h for bacteria and at 25°C for 28 h for fungi. Resazorin indicator solution in 50 μl was added to each well, the microplates were incubated as mentioned before for 30 min and reported, violet color of the contain of the well indicated no bacterial growth, if the color change to pink it indicated microbial growth.

To determine the MBC or MFC, a loop full with 10 μl was removed from the MIC dilution well and from the preceding dilutions, from the negative and positive control wells, all of them were inoculated into MHA or SDA plates and incubated at 37°C for bacteria or 25°C for fungi for 24 h or 48 h respectively, the lower concentration which gave no growth was the required MBC or MFC, and were compared to the MBC or MFC of the positive antimicrobial control [25,26]. All determinations were carried out in triplicate.

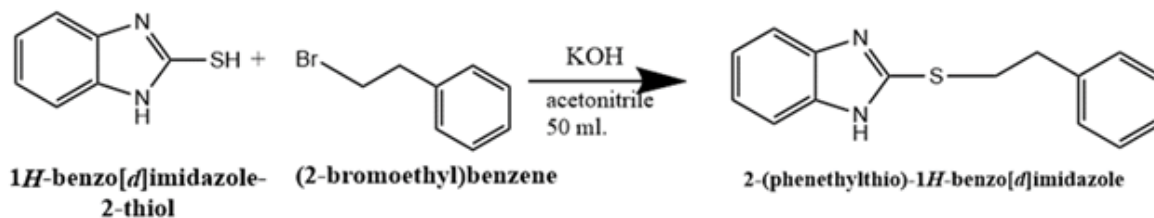
Statistical Analysis

The analysis was carried by using the statistical packages for social science SPSS version 21 for student's t-test. Values are presented as mean \pm SD.

Synthesis

Synthesis of 2-(Phenethylthio)-1H-benzo[d]imidazole (ZR-1): A mixture of 2-mercaptobenzimidazole (1.50 g, 0.01 mol.), potassium hydroxide (0.56 g, 0.01 mol.), acetonitrile 50 ml has been heated and stirred under reflux pressure for 15 minutes until the temperature reach 50°C, the (2-bromoethyl) benzene 2-phenyl-ethyl (1.85 g, 0.01 mol.) was added drop wise, keeping the temperature between (40-50)°C, the reacted mixture was heated and stirred under reflux for 1.30 hours. After cooling, the precipitate filtered and washing with distilled water, white crystal was formed by recrystallization from ethanol-water, finally the product weighted and characterization by DSC, Infrared spectroscopy (FT-IR), $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ (Scheme 1).

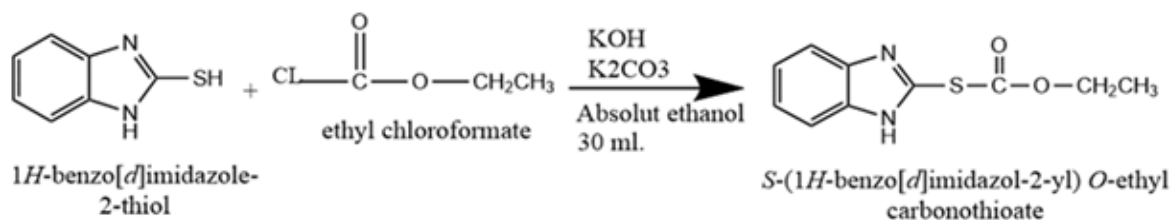
SC: (160°C), FT-IR (KBr): C-H stretching Aromatic (2958.27 cm^{-1}), C=C Stretching Aromatic (1496.49 cm^{-1}), C-N Stretching Aromatic (1268.93 cm^{-1}), C-S Stretching (593.97 cm^{-1}), N-H Stretching (3434.60 cm^{-1}) CH₂ aliphatic (2786.63 cm^{-1}), $^1\text{H-NMR}$: two types of CH₂ proton (3-4 ppm, 3H, triplet), CH of aromatic (7.0 ppm-8.0 ppm), $^{13}\text{C-NMR}$: C11, C12 (35.228-37.74) ppm, C3, C6 (115.090-116.115) ppm, C1, C2 (123.640), C16 (128.088 ppm), C14, C18 (130.036 ppm), C15, C17 (130.036), C4, C5 (141.518 ppm), Elemental analysis: for C₇H₆N₂S: Calcd: C, 70.83%; H, 5.55%; N, 11.01%. Found: C, 70.68%; H, 5.58%; N, 11.22%. MW 254.35.



Scheme 1 Synthesis of 2-(Phenethylthio)-1H-benzo[d]imidazole (ZR-1)

Synthesis of S-(1H-benzo[d]imidazol-2-yl) O-ethyl carbonothioate (ZR-2): A mixture of 2-mercaptobenzimidazole (1.50 g, 0.01 mol.), potassium hydroxide (0.56 g, 0.01 mol.), potassium carbonate (1.38 g, 0.01 mol.), In absolute ethanol 30 ml, heat and stirred under reflux pressure for 5 minutes until the temperature reach (30-40)°C, the ethyl chloroformate (1.08 g, 0.01 mol.) was added drop wise, the mixture was heated and stirred under reflux for 2 hours, after cooling, add water with ice to the mixture than solid white crystal precipitate, filtered, finally the product weighted and characterization by DSC, Inferred spectroscopy (FT-IR), $^1\text{H-NMR}$ and C^{13} NMR (Scheme 2).

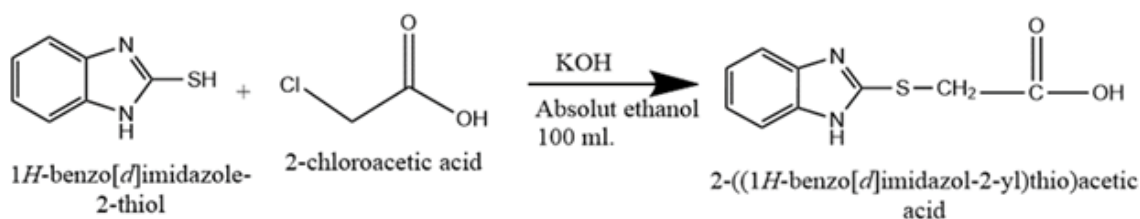
DSC: (160°C), FT-IR (KBr): C-H stretching Aromatic (2983.34 cm^{-1}), C=C Stretching Aromatic (1606.34 cm^{-1}), C-N Stretching Aromatic (1322.93 cm^{-1}), C-S Stretching (626.68 cm^{-1}), N-H Stretching (3453.88 cm^{-1}), $^1\text{H-NMR}$: CH3 protons (1.38 ppm, 3H, triplet), CH2 protons (3.344 ppm, 2H, multiplet), Two types of aromatic proton (7.0 ppm-8.0 ppm), $^{13}\text{C-NMR}$: C15 (16.778 ppm), C14 (55.822 ppm), C3, C6 (106.966 ppm), C1, C2 (125.003 ppm), C8 (146.163 ppm), C11 (163.527 ppm), Elemental analysis: for $\text{C}_{10}\text{H}_{10}\text{N}_2\text{O}_2\text{S}$: Calcd: C, 54.04%; H, 4.54%, N, 12.60%.



Scheme 2 Synthesis of S-(1H-benzo[d]imidazol-2-yl) O-ethyl carbonothioate (ZR-2)

Synthesis of 2-((1H-benzo[d]imidazol-2-yl) thio) acetic acid (ZR-3): A mixture of 2-mercaptobenzimidazole (1.50 g, 0.03 mol.), potassium hydroxide (2 g, >0.03 mol.) in 100 ml in absolute ethanol heat with stirring under reflux, then 2-chloroacetic acid (3.5g, >0.03 mol.) was added dropped wise with continues stirring and heat up to 60°C for 4 hours. The mixture was cooled to room temperature and added water then formed white foam on the surface, filtered and dry in oven at temperature 50°C for 30 minutes. The precipitate weighted and characterized by DSC, $^1\text{H NMR}$, $^{13}\text{C NMR}$, and FI-IR (Scheme 3).

DSC: (230°C), FT-IR (KBr): C-H Stretching Aromatic (2915.84 cm^{-1}), C=C Stretching Aromatic (1573.63 cm^{-1}), C-S Stretching (605.54 cm^{-1}), N-H Stretching (3430.74 cm^{-1}), $^1\text{H-NMR}$: CH2 proton (3.156 ppm, singlet), CH of aromatic (7-8) ppm, $^{13}\text{C-NMR}$: C11 (36.303), C6, C3 (115.622), C1, C2 (123.974), C4, C5 (140.101), C8 (163.527), C13 (172.934), Elemental analysis: for $\text{C}_9\text{H}_8\text{N}_2\text{O}_2\text{S}$: Calcd: C, 51.91%; H, 3.87%, N, 13.45%. Found: C, 52.02%, H, 3.75%; N, 13.53%. MW 208.03.

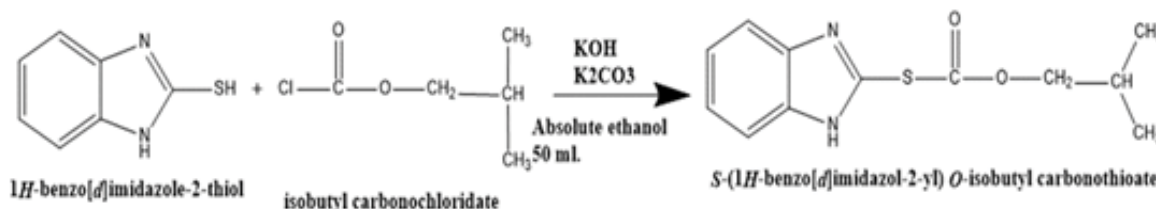


Scheme 3 Synthesis of 2-((1H-benzo[d]imidazol-2-yl) thio) acetic acid (ZR-3)

Synthesis of S-(1H-benzo[d]imidazol-2-yl) O-isobutyl carbonothioate (ZR-4): A mixture of 2-mercaptobenzimidazole (1.50199 g, 0.01 mol.), K_2CO_3 (1.38 g, 0.01 mol.), KOH (0.56 g, 0.01), in 50 ml absolute ethanol, heated and stirred for 45 minutes at 60°C. Cool to room temperature and add isobutyl chloroformate (0.01 mol, 1.36 ml) dropped wise the mixture heated to 45°C under reflex for 2 hours. The mixture cool to room temperature and poured in to 100 ml of iced water. Precipitate was filtered, recrystallized from ethanol-water. The greenish Product characterized by $^1\text{HNMR}$, $^{13}\text{C NMR}$, DSC, and FT-IR (Scheme 4).

FT-IR (KBr): C-H stretching Aromatic (2964.05 cm^{-1}), C=C Stretching Aromatic (1621.84 cm^{-1}), C-N Stretching Aromatic (1326.79 cm^{-1}), C-S Stretching (628.68 cm^{-1}), N-H Stretching (3110.62 cm^{-1}), Ester stretching (1737.55 cm^{-1}), $^1\text{H-NMR}$: Two types CH3 protons (2.504 ppm, 6H, doublet), CH proton (3.251 ppm, 1H, multiplet), CH2 aliphatic (4.617 ppm, 2H, doublet), CH of aromatic protons (7.0 ppm-8.0 ppm, multiplet), $^{13}\text{C-NMR}$: C16, C17 (20.5 ppm),

C15 (36.24 ppm), C14 (72.72 ppm), C3, C6 (109.418), C1, C2 (122.246), C4, C5 (130.990), C8 (159.19 ppm), C11 (165.93 ppm), Elemental analysis: for $C_9H_8N_2O_2S$: Calcd: C, 57.58%; H, 5.64 %; N, 11.19%, Found: C, 57.55%; H, 5.63%; N, 11.25%. MW 250.32.



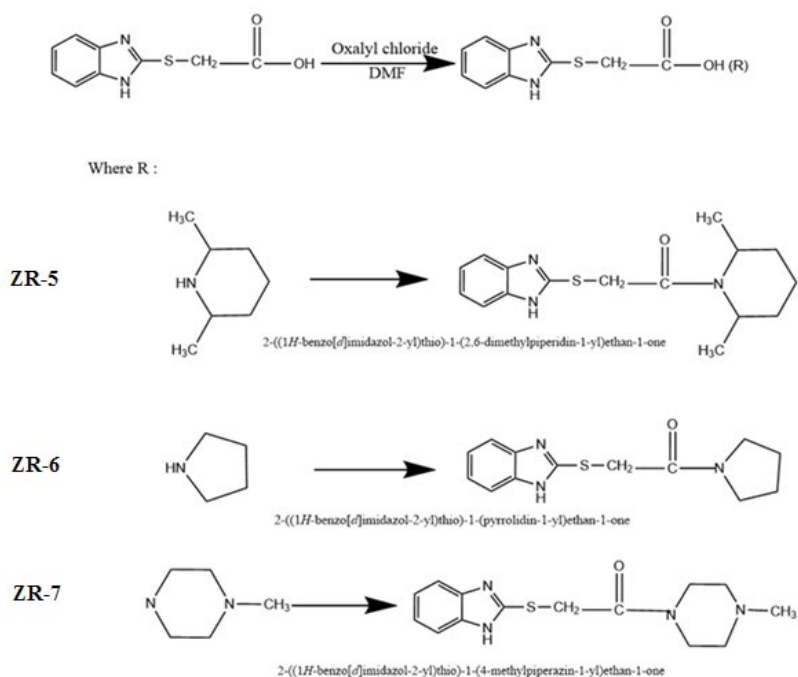
Scheme 4 Synthesis of S-(1H-benzo[d]imidazol-2-yl) O-isobutyl carbonothioate (ZR-4)

Synthesis of 2-((1H-benzo[d]imidazol-2-yl) thio) acetic acid derivatives (ZR-5, ZR-6, ZR-7): A mixture of (ZR-3) (2.08 g, 0.01 mol.), oxalyl chloride (0.8 ml), few drops of dimethyl formamide, in dichloromethane (50 ml), has been heated and stirred under reflux for 18 hours at 25°C. Added appropriate amount of: 2, 6-dimethyl piperidine, Pyrrolidine, N-methyl piperazine drop wise and stirred at room temperature for 18 hours under reflux. The precipitate was filtered, wash with little amount of distilled water generate different colors, orange (ZR-5), green (ZR-6), orange (ZR-7). Different products confirm through $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, DSC, and FT-IR (Scheme 5).

2-((1H-benzo[d]imidazol-2-yl) thio)-1-(2, 6-dimethylpiperidin-1-yl) ethan-1-one (ZR-5): DSC: (165-170)°C, FT-IR (KBr): C-H stretching Aromatic (2965.98cm^{-1}), C=C Stretching Aromatic (1508.06cm^{-1}), C-N Stretching Aromatic (1373.07cm^{-1}), C-S Stretching (698.11cm^{-1}), N-H Stretching (3133.76cm^{-1}), CH₃ (2923.56) stretching aliphatic, $^1\text{H-NMR}$: Two types of CH₃ protons (1.0 ppm-2.0 ppm, 3H, multiplet), various proton on cyclic amine (2.5 ppm-3.5ppm, multiplet), two types of CH₂ protons (3.75 ppm-4.0ppm, 2H, multiplet), CH₂ proton (4.67ppm, 2H, S-CH₂, singlet), CH of aromatic (7-8) ppm, $^{13}\text{C-NMR}$: C20, C21 (18.876), C17 (22.061), C16, C18 (29.560), C19, C15 (37.384-42.936), C11 (52.223), C6, C3 (111.796), C1, C2 (118.503), C5, C4 (123.421), C8 (149.287), C12 (164.300), Elemental analysis: for $C_{16}H_{21}N_3OS$: Calcd: C, 63.34%; H, 6.98%; N, 13.85%, Found: C, 63.55%, H, 6.95%, N, 13.55%. M.wt = 303.42.

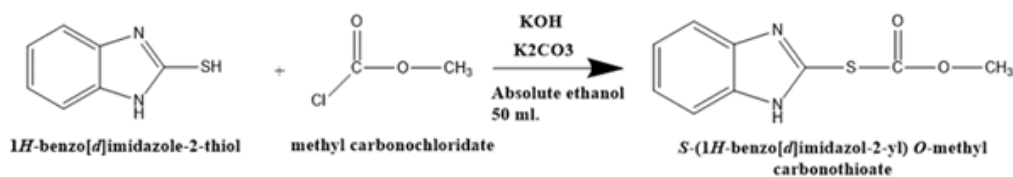
2-((1H-benzo[d]imidazol-2-yl) thio)-1-(pyrrolidin-1-yl) ethan-1-one (ZR-6): FT-IR (KBr): C-H stretching Aromatic (3000cm^{-1}), C=C Stretching Aromatic (1712.48cm^{-1}), C-N Stretching Aromatic (1330.64cm^{-1}), C-S Stretching (644.11cm^{-1}), N-H Stretching (3100.0cm^{-1}), Ketone stretching (1754.90cm^{-1}), $^1\text{H-NMR}$: Various proton of cyclic amine (2.5ppm- 4.0 ppm, multiplet), CH₂ aliphatic (4.75ppm, 2H, singlet), CH of aromatic (7-8 ppm) (Figure 3.6)., $^{13}\text{C-NMR}$: C15, C16 (23.108), C18 (34.661), C14, C17 (39.467), C14, C17 (45.021), C3, C6 (11.801), C1, C2 (113.198), C4, C5 (118.494), C8 (149.919), C11 (168.743), Elemental analysis: for $C_{13}H_{15}N_3OS$: Calcd: C, 59.75%; H, 5.79%; N, 16.08%. M.wt = 261.34.

2-((1H-benzo[d]imidazol-2-yl) thio)-1-(4-methylpiperazin-1-yl) ethan-1-one (ZR-7): FT-IR (KBr): C-H stretching Aromatic (2923.56cm^{-1}), C=C Stretching Aromatic (1500.06cm^{-1}), C-N Stretching Aromatic (1303.64cm^{-1}), C-S Stretching (698.11cm^{-1}), N-H Stretching (3396.03cm^{-1}), Ketone stretching (1739.48cm^{-1}), C-N tertiary amine(1265.07cm^{-1}), CH₃ Stretching (3000.0cm^{-1}), $^1\text{H-NMR}$: Various protons of cyclic methyl piperazine (2.0 ppm – 4.0 ppm, multiplet), CH₂ aliphatic (4.75ppm, 2H, singlet), CH of aromatic (7.0 ppm-8.0 ppm, multiplet), $^{13}\text{C-NMR}$: C11 (40.026), C20 (42.360), C15, C9 (49.130), C18, C16 (51.870), C3, C6 (113.209), C1, C2 (118.499), C4, C5 (123.957), C8 (129.743), C13 (165.937), Elemental analysis: for $C_{14}H_{18}N_4OS$: Calcd: C, 57.91%; N, 6.25 %; N, 19.29%. M.wt = 290.39.



Scheme 5 Synthesis of 2-((1H-benzo[d]imidazol-2-yl) thio) acetic acid derivatives

Synthesis of S-(1H-benzo[d]imidazol-2-yl) O-methyl carbonothioate (ZR-8): A mixture of 2-mercaptobenzimidazole (1.50 g, 0.01 mol.), potassium hydroxide (0.56 g, 0.01 mol.), potassium carbonate (1.38 g, 0.01 mol.), Absolute ethanol 50 ml. Heated and stirred for 30 minutes until the temperature reach 50°C, the methyl chloroformate (0.945 g, 0.01 mol.) was added drop wise. Keeping the temperature between (40-50)°C for 30 minutes. The reaction was cooled to room temperature and the white mixture filtrated and concentrated under reduces pressure. White crystals formed, recrystallized in ethanol-water. The product weighted and the structure was confirmed by DSC, Inferred spectroscopy (FT-IR), ¹H-NMR and ¹³C-NMR (Scheme 6).



Scheme 6 Synthesis of S-(1H-benzo[d]imidazol-2-yl) O-methyl carbonothioate (ZR-8)

S-(1H-benzo[d]imidazol-2-yl) O-methyl carbonothioate (ZR-8): FT-IR (KBr): C-H stretching Aromatic (3100.0 cm^{-1}), C=C Stretching Aromatic (1617.98 cm^{-1}), C-N Stretching Aromatic (1330.64 cm^{-1}), C-S Stretching (761.74-599.75) cm^{-1} , N-H Stretching (3149.19 cm^{-1}), Thioester stretching (1733.69 cm^{-1}), ¹H-NMR: CH₃ proton (2.5ppm, 3H, singlet), CH of aromatic (7-8) ppm, ¹³C-NMR: C14 (39.490), C3, C6 (109.723), C1, C12 (119.004), C4, C5 (149.267), C8 (165.936), Elemental analysis: for C₉H₈N₂O₂S; Calcd: C, 51.91%; H, 3.87%; N, 13.45%. M.WT=208.03 g/mol.

RESULTS AND DISCUSSION

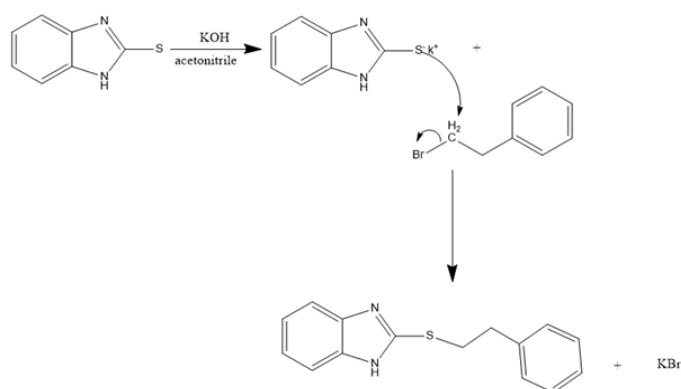
Chemistry

The designed compounds were prepared as shown in scheme 1. Compound 2-(Phenethylthio)-1H-benzo[d]imidazole (ZR-1) was prepared from 2-MBI with 2-bromoethyl benzene in presence basic acetonitrile (K₂CO₃). The reaction involves direct displacement by the ionic sulfur on the 2-bromoethyl benzene. Methyl, ethyl and isobutyl chloroformate were reacted with 2-mercaptobenzimidazole in the presence of absolute ethanol with K₂CO₃, the reaction proceeds through direct displacement of ionic sulfur on the chloroformate. Generating compounds (ZR-2, ZR-4, and

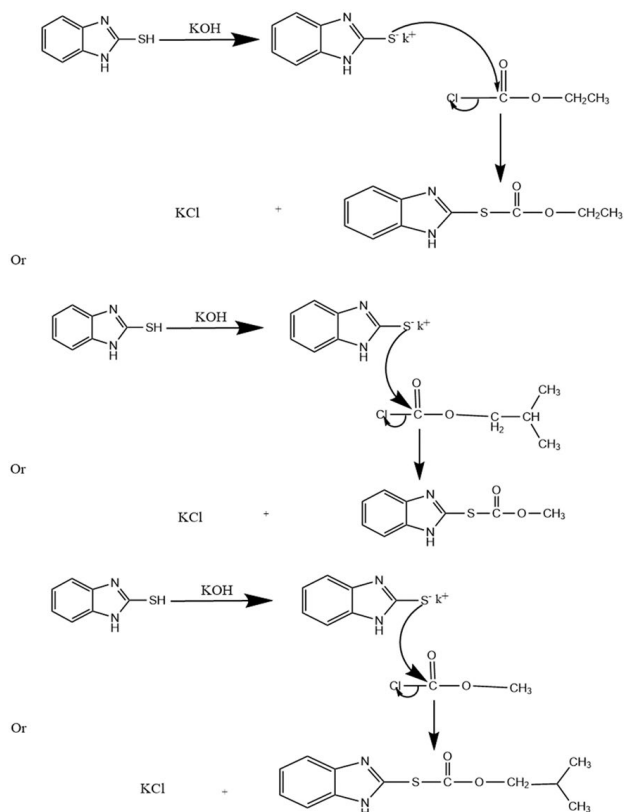
ZR-8). 2-mercaptobenzimidazole treated with chloroacetic acid. Amid formation of compounds (ZR-5, ZR-6, and ZR-7) generate through reaction of compound ZR-3 were converted to corresponding acyl derivatives addition of the piperidine, N-Methyl Piperazine, Pyrrolidine lead to formation of compounds (ZR-5, ZR-6, ZR-7). The mechanism of the acylation by oxalyl chloride is outline in Schemes 7-10. All compounds prepared were consistent with DSC, IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and elemental analysis.

Molecular Modelling

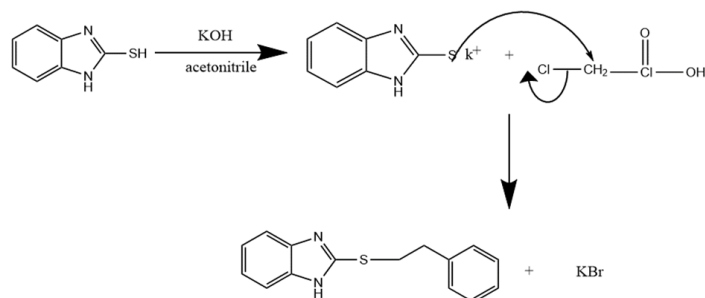
Molecular docking (Table 1) indicate the affinity binding of ZR-5 is inhibiting dihydro reductase enzyme greater than corresponding the reference trimethoprim whereas (8.9 kcal/mol) relative to (7.9 kcal/mol) for reference (Table 2) (Figure 3). The important residues (thioacetate 2, 6 dimethyl piperidine) in different conformation are overlap with the enzyme DHFR to induce greater affinity, interacting with Val 8, Ala 9, Ser 59 and Glu 30 most of other ligands. The ligands also interact with NADPH oxygen residue. The interaction binding energy showed that ZR-4 has higher interactions and good binding energy.



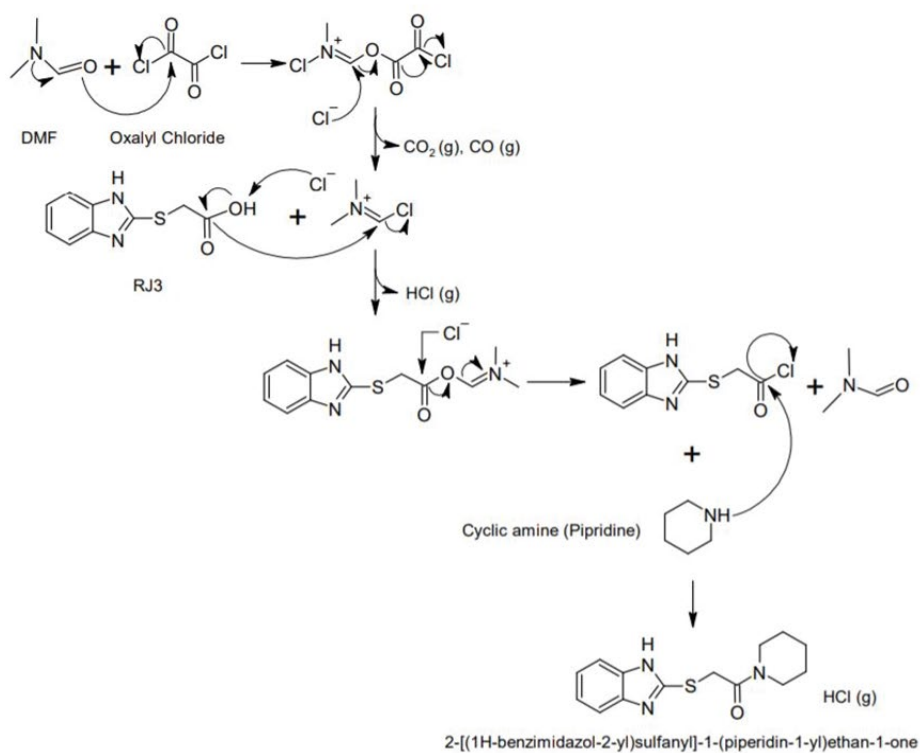
Scheme 7 The mechanism of the acylation by oxalyl chloride



Scheme 8 The mechanism of the acylation by oxalyl chloride



Scheme 9 The mechanism of the acylation by oxalyl chloride



Scheme 10 The mechanism of the acylation by oxalyl chloride

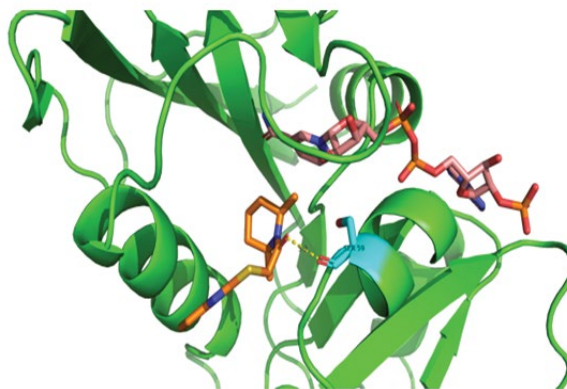


Figure 3 ZR-5 docking with NADPH complex

Table 1 Docking Results of test compounds into the DHFR binding sites

Compound code	Binding Energy (in kJ/mol)	Log P (o/w)	molecular weight g/mol.
STANDER	-7.91	0.91	290
ZR-1	-8.20	4.42	254.35
ZR-2	-6.62	2.87	222.26
ZR-3	-7.20	1.5	208.03
ZR-4	-7.30	3.75	250.32
ZR-5	-8.70	2.69	303.42
ZR-6	-7.60	1.63	261.34
ZR-7	-8.00	1.07	290.39
ZR-8	-7.00	2.53	208.03

Table 2 ZR-5 interactions with DHFR binding site

Ligand	Ligand no.	Atom name	chain	Atom no.	Residue	Residue no.	Atom name	chain	M. mass	Distance (in Å)
ZR-5	1	O32	A	63	SER	59	O	A	462	3.30793

Antimicrobial Activity:

The antimicrobial activity of the synthesized derivatives compounds (ZR-1 to ZR-8), were evaluated by agar diffusion and broth dilution methods. According to agar diffusion method the zone of inhibition results, showed antibacterial activities but were less than the standard control. All the synthesized derivatives (ZR-1 to ZR-8) had antifungal activity against *C. albicans* with a zone of inhibition diameter ranging from (15-18) mm (Table 3).

Table 3 The zone of inhibition diameter in mm of the synthesized compounds at 500 µg/ml concentration

Microorganism	<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
Concentration Compound	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml	500 µg/ml
ZR-1	8 ± 2.8	12 ± 1.2	8 ± 2.7	9 ± 1.2	15 ± 1.8
ZR-2	8 ± 2.1	10 ± 1.6	6 ± 1.8	10 ± 1.6	15 ± 1.8
ZR-3	6 ± 1.8	11 ± 1.4	6 ± 1.8	12 ± 1.2	14 ± 2.0
ZR-4	10 ± 1.6	13 ± 2.1	7 ± 2.2	11 ± 1.4	17 ± 1.8
ZR-5	7 ± 2.1	10 ± 1.6	5 ± 2.6	10 ± 1.6	16 ± 1.4
ZR-6	5 ± 2.5	12 ± 1.8	6 ± 1.8	13 ± 2.1	15 ± 2.1
ZR-7	7 ± 2.1	10 ± 1.6	6 ± 1.6	10 ± 1.8	16 ± 1.4
ZR-8	10 ± 1.6	15 ± 1.5	9 ± 1.2	14 ± 2	18 ± 1.7
Miconazole (500 µg/ml)	-	-	-	-	15 ± 1.2
Ciprofloxacin (5 µg/ml)	16 ± 1.5	22 ± 1.5	24 ± 2	20 ± 1.8	-
Trimethoprim (200 µg/ml)	10 ± 1.8	11 ± 1.6	8 ± 2.8	9 ± 1.7	-
Negative control	-	-	-	-	-

Values are mean ± SD (n-3), -: no effect; **ZR-1:** 2-(Phenethylthio)-1H-benzo[d]imidazole; **ZR-2:** S-(1H-benzo[d]imidazol-2-yl) O-ethyl carbonothioate; **ZR-3:** 2-((1H-benzo[d]imidazol-2-yl) thio) acetic acid; **ZR-4:** S-(1H-benzo[d]imidazol-2-yl) O-isobutyl carbonothioate; **ZR-5:** 2-((1H-benzo[d]imidazol-2-yl) thio)-1-(2, 6-dimethylpiperidin-1-yl) ethan-1-one; **ZR-6:** 2-((1H-benzo[d]imidazol-2-yl) thio)-1-(pyrrolidin-1-yl) ethan-1-one; **ZR-7:** 2-((1H-benzo[d]imidazol-2-yl) thio)-1-(4-methylpiperazin-1-yl) ethan-1-one; **ZR-8:** S-(1H-benzo[d]imidazol-2-yl) O-methyl carbonothioate

When the antimicrobial activity of the synthesized derivatives compounds (ZR-1 to ZR-8) was evaluated by detection of MIC and MBC/MFC, the activity was higher as demonstrated in Table 4.

Table 4 The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC)/Minimum Fungicidal Concentration (MFC) of the synthesized compounds (ZR-1 to ZR-8) in µg/ml against *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans*

Microrganism	<i>E. coli</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
Concentration Compound	MIC/MBC (µg/ml)	MIC/MBC (µg/ml)	MIC/MBC (µg/ml)	MIC/MBC (µg/ml)	MIC/MFC (µg/ml)
ZR-1	15.62/15.62	15.62/31.25	62.5/125	31.25/62.5	62.5/125
ZR-2	31.25/62.5	62.5/125	62.5/125	62.5/125	31.25/62.5
ZR-3	62.5/125	15.62/31.25	62.5/125	31.25/62.5	62.5/125
ZR-4	62.5/125	15.62/31.25	62.5/125	31.25/62.5	15.62/31.25
ZR-5	62.5/125	15.62/31.25	62.5/125	31.25/62.5	31.25/62.5
ZR-6	62.5/125	62.5/125	62.5/125	62.5/125	31.25/62.5
ZR-7	62.5/125	15.62/31.25	62.5/125	31.25/62.5	31.25/62.5
ZR-8	15.62/31.25	31.25/62.5	62.5/125	31.25/62.5	3.40/7.81
Miconazole (500 µg/ml)	-	-	-	-	31.25/62.5
Ciprofloxacin (5 µg/ml)	62.5/125	62.5/125	15.6/31.25	125/250	31.25
Trimethoprim (200 µg/ml)	250/500	62.5/125	250/500	125/250	-
Negative control	-	-	-	-	-

ZR-1: 2-(Phenethylthio)-1H-benzo[d]imidazole; ZR-2: S-(1H-benzo[d]imidazol-2-yl) O-ethyl carbonothioate; ZR-3: 2-((1H-benzo[d]imidazol-2-yl) thio) acetic acid ; ZR-4: S-(1H-benzo[d]imidazol-2-yl) O-isobutyl carbonothioate; ZR-5: 2-((1H-benzo[d]imidazol-2-yl) thio)-1-(2, 6-dimethylpiperidin-1-yl) ethan-1-one; ZR-6: 2-((1H-benzo[d]imidazol-2-yl) thio)-1-(pyrrolidin-1-yl) ethan-1-one; ZR-7: 2-((1H-benzo[d]imidazol-2-yl) thio)-1-(4-methylpiperazin-1-yl) ethan-1-one; ZR-8: S-(1H-benzo[d]imidazol-2-yl) O-methyl carbonothioate; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration, MFC: Minimum Fungicidal Concentration ; -: No activity

The newly synthesized compounds (ZR-1 to ZR-8) showed higher antimicrobial activity against the tested microorganisms, as evaluated by detection of the MIC and MBC or MFC than the activity detected using agar diffusion method which was also reported by other [27], the variability of the results may be due to their general reactivity, resulted in a low antimicrobial activity against *B. subtilis*, which possess endospore, and of their low solubility in water that made their diffusion in agar medium weak. Compounds ZR-8 exhibited the highest antibacterial activity against *E. coli*, *S. aureus*, *P. aeruginosa* with MIC/MBC of 15.62/31.25, 31.25/62.5, 31.25/62.5 respectively which were lower than that of ciprofloxacin (5 µg/ml) and trimethoprim (200 µg/ml), compound ZR-1 exhibited good activity against *E. coli* and *S. aureus* with MIC/MBC of 15.62/31.25, compounds ZR-3, ZR-4, ZR-5 and ZR-7 showed good activity against *S. aureus* with MIC/MBC of 15.62/31.25. The antibacterial activity of all compounds (ZR-1 to ZR-8) against *P. aeruginosa* was better than the standard control, which may be attributed to the good permeability of these compounds through the microorganism outer membrane by dual pathway [27]. ZR-8 exhibited excellent activity against *C. albicans* with MIC/MFC of 3.40/ 7.81 which was lower than miconazole (500 µg/ml) with MIC/MFC of 31.25/62.5, followed by ZR-4 with MIC/MFC of 15.62/31.25, ZR-2, ZR-5, ZR-6 and ZR-7 showed the same antifungal activity similar to miconazole as shown in table 4. Due to their structural similarities with the purine the suggested mechanism of action for the antimicrobial activity is by inhibiting the microbial nucleic acid and proteins synthesis, [12], and for antifungal selectivity of these derivatives (ZR-1 to ZR-8) was through inhibition of CA-CYP51 resulted in blocking ergosterol formation, thus facilitate permeability through the cell membrane [28].

CONCLUSION

The approach for this synthesize derivatives of 2-mercaptobenzimidazole and elucidation by NMR, FT-IR of their structures was consistent with the design compounds. The compound ZR-5 (2-((1H-benzo[d]imidazol-2-yl) thio)-1-(2, 6-dimethylpiperidin-1-yl) ethan-1-one) its shows higher affinity for DHFR. Compound (ZR-8) showed excellent antifungal activity in comparison with miconazole and good antibacterial activity compared to ciprofloxacin and trimethoprim.

DECLARATIONS

Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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