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Synthesis, Characterization and Antimicrobial Evaluation of New 5-Methoxy-2-Mercapto Benzimidazole Derivatives

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Abstract:

A novel series of 5-methoxy-2-mercapto benzimidazole derivatives **1-8**, were successfully synthesized. The reactions were achieved through N, and S-alkylation of the parent nucleus, and also Mannich reaction.

The newly synthesized compounds were elucidated and confirmed utilizing the corresponding analytical and spectroscopic data; including FT-IR, ^{1}HNMR, and CHN analysis. All the title compounds were evaluated for their *in vitro* preliminary antimicrobial activities against six bacterial strains, *Staphylococcus aureus* ATCC: 6538, *Bacillus subtilis* ATCC: 6051, *Bacillus pumilus* NCTC: 8241**,** *Pseudomonas aeruginosa* ATCC: 9027, *Escherichia coli* ATCC: 8739, *Enterobacter cloacae* ATCC: 13047, and three fungal strains, *Aspergillus Niger* ATCC:1015, *Penicillium expansum* ATCC: 7861 *and Candida albicans* ATCC: 10231, by Well diffusion method. The title compounds showed moderate to high specific antibacterial activities against (*Escherichia coli* and *Enterobacter cloacae*) and the compounds **1- 4, 10** and **13** exhibited specific and most potent antibacterial activity against *Enterobacter cloacae* at concentration 100 µg/ml, using cefotaxime and sulphamethoxazole as standard antibacterial drugs.

While the compounds **12** and **13** exhibited the highest antifungal activity against *Aspergillus niger*, using miconazole as a standard antifungal drug.

Keywords: Antimicrobial activity, benzimidazole derivatives, Mannich reaction, 5-methoxy-2-mercapto benzimidazole.

1.INTRODUCTION

Benzimidazole derivatives are of wide significance because of their various biological activities and clinical application [1].This parent nucleus is found in different antiparasitic, fungicidal, antihelmintic and anti-inflammatory drugs[2-5].

Optimization of the substituent around the benzimidazole nucleus has resulted in many drugs as antihelminthics like albendazole, mebendazole, thiabendazole; proton pump inhibitors, like omeprazole, lansoprazole, pantoprazole, and many other compounds in the field of medicinal and therapeutic area[6].

The structural modification, of the benzimidazole moiety, can be carried out to all seven positions of the ring, with different chemical functional groups, but most of the biologically active benzimidazole-based compounds bear different entities at 1,2 and /or 5(or 6) positions.

There is still an interest in the synthesis of substituted 2-mercapto benzimidazole derivatives for obtaining newly active biological compounds, our earlier report on 5-methoxy-2-mercapto benzimidazole derivatives as anticonvulsant drugs,[7] is a quite dynamic , affording many promising research challenges.In continuation to our work on 5-methoxy-2-mercaptobezimidazole, the present work focuses on the synthesis of different derivatives at 1,2-positions of the parent nucleus and then to screen them for their antimicrobial activity.

2. MATERIALS AND METHODS

All melting points were measured by an electric melting point apparatus (Thomas Hoover UK).This apparatus was used to determine all melting points reported and were used uncorrected. Determinations of infrared (IR) spectra were recorded using KBr disc on a Shimadzu Spectrophotometer (υ_{max} in cm^{-1}) (WQF-520, Japan). The proton ^{1}HNMR spectra were recorded on (Bruker, Germany NMR Spectrometer 400 MHz, Avance III 400 spectrometer) with tetramethylsilane (TMS) as an internal standard, (δ=ppm) and coupling constant in Hz, which was run in (Central Laboratory Isfahan University)-IRAN.

The CHN elemental microanalysis of the synthesized final products was done by the micro analyzer ((Euro EA3000, Europe). The purity of the synthesized compounds and the progress of the reaction were determined by Thin Layer Chromatography on aluminum silica gel 60 F_{254} (Merck) detected by UV (ultraviolet) light (254 nm). All the chemicals purchased were of analytical grade and were used without further purification unless otherwise stated.The synthetic method is depicted in Scheme 1, and the physical data of the synthesized compounds are listed in table 1.

2.1. General method for the Synthesis compounds (1- 4) [8]

5-methoxy-2-MBI,(0.05 mol, 9.01 g) was dissolved in absolute ethanol (15 ml) with each of the four different alkyl halides (0.05 mol, 4.74g methyl bromide, 5.44g ethyl bromide, 4.14g propyl bromide and 6.85g *sec*-butyl bromide) and sodium hydroxide (0.05 mol,2.0g) in round flask (50 ml) and a reflux condenser. The mixture was refluxed for 7h and filtered directly to get rid of the precipitated salt, the filtered sample was cooled and recrystallized from ethanol and water.

Column chromatography was run using S.G (60-120 mesh) and the mobile phase (*n*-hexane: ethyl acetate (7.5:2.5) for compound **1**, Chloroform: acetone (9.5: 0.5) for compound **2**, Chloroform: acetone (9.0: 1.0) for compound **3** and *n*-

hexane: ethyl acetate (8.0:2.0) for compound **4** to purify the titled products.

Synthesis of 5-methoxy-2-(methylthio)-1*H*-benzo[d]imidazole. (1)
Off-white to beige powder, yield: 93%; m.p. 125-128°C; **IR (υ=cm^{-1},KBr)**: 3153 (υ N-H)str, 3047(υ Ar-H)str, 2997 and 2949 (υ aliph.CH_3 a*sym.* and *sym.*)str, 1633 (υ C=N)str, 1595(υ (Ar-C=C)str, 1539 (υ N-H) bend., 1267 and 1031 (υ C-O methyl ether)str, 642(υ C-S)str; **^{1}HNMR (DMSO-d_6, δ ppm)**: 7.33 (d,1H,Ar-H_7), 6.97(d,1H, Ar-H_4),6.74 (dd,1H,Ar-H_6),3.76 (s,3H,OCH_3), 2.67 (s,3H,CH_3-S); Elemental analysis Calcd. for $C_9H_{10}N_2OS$: C,55.65; H,5.19; N,14.42; S,16.51. Found:C,55.54; H,5.394; N,14.649; S,15.55.

Synthesis of 2-(ethylthio)-5-methoxy-1*H*-benzo[d]imidazole (2)
Off-white to beige powder, yield: 72%; m.p. 146-149°C; **IR (υ= cm^{-1},KBr)**:
3149 (υ N-H)str, 3047(υ Ar-H)str, 2997 and 2879 (υ aliph. CH_3 a*sym.* and *sym.*)str, 2953 and 2827 (υ aliph. CH_2 a*sym.* and *sym.*)str,1635(υ C=N)str, 1595(υ Ar-C=C)str, 1267and 1033 (υ C-O methyl ether)str, 690(υ C-S)str; **^{1}HNMR (DMSO-d_6, δ ppm)**: 7.34 (d,1H,Ar-H_7), 6.98 (d,1H,Ar-H_4), 6.76 (dd,1H,Ar-H_6), 3.77 (s,3H,OCH_3), 3.00 (q,2H,CH_2), 1.22 (t,3H,CH_3); Elemental analysis Calcd. for $C_{10}H_{12}N_2OS$: C,57.67; H,5.81; N,13.45; S,15.40. Found:C,57.852; H,5.68; N;13.68; S,15.21.

Synthesis of 5-methoxy-2-(propylthio)-1*H*-benzo[d]imidazole (3)
Beige powder, yield: 76%; m.p. 60-61°C; **IR (υ= cm^{-1},KBr)**: 3304 (υ N-H)str, 3062 (υ Ar-H)str, 2960 and 2887 (υ aliph. CH_3 a*sym.* and *sym.*)str, 2835 (υ aliph. CH_2 *sym.*)str, (υ *asym.* str. was overlapped with that of alph, (CH_3)str, 1622(υ C=N)str, 1509 (υ Ar-C=C)str, 1265 and 1026 (υ C-O methyl ether)str, 624(υ C-S)str; **^{1}HNMR (DMSO-d_6, δ ppm)**: 7.32 (d,1H,Ar-H_7), 6.97 (d,1H, Ar-H_4), 6.74 (dd,1H, Ar-H_6), 3.76 (s,3H, OCH_3), 3.21 (t,2H, CH_2), 1.71 (m,2H, CH_2), 0.99 (t,3H,CH_3); Elemental analysis Calcd. for $C_{11}H_{14}N_2OS$: C,59.43; H,6.35; N,12.60; S,14.42.Found:C,59.33; H,6.12; N,11.92; S,13.96..

Synthesis of 2-(*sec*-butylthio)-5-methoxy-1*H*-benzo[d] imidazole (4)
Off-white powder, yield: 76%; m.p. 79-82°C; **IR (υ=cm^{-} 1,KBr)**: 3049 (υ Ar-H)str, 2962 and 2873 (υ aliph. CH_3 a*sym.* and *sym.*)str, 2929(υ aliph. CH_2 a*sym.*)str, (υ *sym.* was overlapped with that of aliph.CH_3)str, 1631(υ C=N)str, 1591(υ Ar C=C)str, 1274 and 1028 (υ C-O methyl ether)str, 630(υ C-S)str; **^{1}HNMR (DMSO-d_6, δ ppm)**: 7.35 (d,1H,Ar-H_7), 6.98 (d,1H, Ar-H_4), 6.75 (dd,1H, Ar-H_6), 3.76 (s,3H,OCH_3), 2.7 (m,1H,CH), 1.68 (m,2H,CH_2), 1.36 (d,3H,($\underline{CH_3}$-CH), 0.97 (t,3H,CH_3); Elemental analysis Calcd. for $C_{12}H_{16}N_2OS$: C,60.99; H,6.82; N,11.85; S,13.57. Found: C, 60.73; H,6.95; N,11.29; S,13.16.

2.2.General method for the synthesis of compounds (5-7) [9]
5-methoxy-2-MBI,(0.01 mol,1.80 g) was dissolved in absolute methanol (15 ml) with each of the desired compounds, having secondary amine (0.01 mol, 0.73g diethylamine, 0.87g morpholine and 0.85g piperidine) mixed in a beaker under the perfect ice-cold condition and stirred constantly. To this solution, an excess formaldehyde (38 %, 0.05mol, 2.6 ml) was added slowly and heated to reflux for 3 h. The content was kept overnight in the freezer. The corresponding crystals obtained, was recrystallized from ethanol.

Synthesis of N-ethyl-N-((5-methoxy-1*H*-benzo[d] imidazole-2-yl)thio) methyl) ethanamine (5)
Off-white powder, yield: 75%; m.p. 135-137°C; **IR (υ=cm^{-} 1,KBr)**: 3300 (υ NH)str, 3109 (υ Ar-H)str, 2945 and 2831 (υ aliph. CH_3 a*sym.* and *sym.*)str, 2901(υ aliph. CH_2 a*sym.*)str, (υ *sym.*was overlapped with that of aliph.CH_3)str, 1629 (υ C=N)str, 1496 (υ Ar-C=C)str, 1274 and 1035(υ C-O methyl ether)str,1163(υ C-N)str, 628(υ C-S)str; Elemental analysis Calcd. for $C_{13}H_{19}N_3OS$: C,58.84; H,7.22; N,15.83;S,12.08. Found: C,58.42; H,6.92; N,15.33; S,12.71.

Synthesis of 4-[(5-methoxy-1*H*-benzo[d]imidazole-2-yl)thio) methyl] morpholine (6)
Beige powder, yield: 73%; m.p. 147-151°C; **IR (υ=cm^{-} 1,KBr)**: 3385(υ NH)str, 3115 (υ Ar-H)str, 2962 and 2835 (υ aliph.CH_3 a*sym.* and *sym.*)str, 2906(υ aliph. CH_2 a*sym.*)str, (υ *sym.* was overlapped with that of aliph. CH_3)str, 1625(υ C=N)str, 1550 (υAr-C=C)str, 1274 and 1035(υ C-O methyl ether)str, 1163(υ C-N)str, 1122(υ C-O-C morpholine)str, 632(υ C-S)str; **^{1}HNMR (DMSO-d_6, δ ppm)**:7.40 (d,1H,Ar-H_7), 7.14 (d,1H,Ar-H_4),7.05 (dd,1H,Ar-H_6), 3.81 (s,2H, CH_2-N), 3.75 (s,3H,OCH_3), 4.61 (td,2H, O(CH_2) morpholine), 3.77 (dd,2H,O-(CH_2) morpholine), 3.54 (d,2H, N(CH_2) morpholine),2.65(td,2H,N-(CH_2)morpholine); Elemental analysis Calcd. for $C_{13}H_{17}N_3O_2S$: C,55.89; H,6.13; N,15.04;S,11.48. Found: C,54.92; H,5.98;N,14.69; S,12.09.

Synthesis of 5-methoxy-2-(piperidine-1-ylmethyl)thio)-1*H*-benzo[d]imidazole (7)
Pale-yellow powder, yield: 65%; m.p. 148-150°C; **IR (υ=cm^{-1},KBr)**: 3385(υ NH)str, 3124 (υ Ar-H)str, 2962 and 2833 (υ aliph. CH_3 a*sym.* and *sym.*)str, 2947 (υ aliph. CH_2 a*sym.*)str, (υ *sym.* was overlapped with that of aliph. (CH_3)str, 1630 (υ C=N)str, 1500 (υ Ar C=C)str, 1274 and 1035 (υ C-O methyl ether)str, 1163 (υ C-N)str, 632 (υ C-S)str; **^{1}HNMR (DMSO-d_6, δ ppm)**: 7.39 (d,1H,Ar-H_7), 7.14 (d,1H,Ar-H_4),7.06 (dd,1H, Ar-H_6), 3.81 (s,2H,CH_2-N), 3.77 (s,3H,OCH_3), 2.68 (m,4H, piperidine), 1.56 (m,4H,piperidine), 1.25 (m,2H,piperidine); Elemental analysis Calcd.for $C_{14}H_{19}N_3OS$: C,60.62; H,6.90; N,15.15;S,11.56. Found: C,59.65; H,6.65; N,14.84; S, 12.05.

2.3. General method for the synthesis of compounds (8-10) [9]

A mixture of equimolar alkaline solution (4N NaOH,0.5 ml) of each compounds **(5-7)**,(0.01 mol), (**5**; 2.65g, **6**;2.79g, and **7**; 2.77g respectively) in methanol (50 ml) and ethyl bromoacetate (0.01mol,1 ml) in methanol (30 ml) was heated gently on boiling water bath for 0.5-1 h. The solid thus obtained on cooling was recrystallized from chloroform.

Synthesis of ethyl 2-(2-((diethylamino)methylthio)-5-methoxy-1*H*-benzo [d]imidazole-1-yl)acetate (8).

Off-white powder, yield: 67%; m.p. 120-123°C; **IR (υ=cm^{-1},KBr)**: 3008(υ Ar-H)str, 2970 and 2818 (υ aliph. CH_3 a*sym.* and *sym.*)str, 2937(υ aliph. CH_2 a*sym.*)str, (υ *sym.* was overlapped with that of aliph. CH_3)str,1730 (υ C=O ester)str, 1635(υ C=N)str, 1504 (υ Ar-C=C)str, 1269 and 1024(υ C-O methyl ether)str, 1199(υ C-O ester)str, 1162(υ C-N)str, 623(υ C-S)str; **^{1}HNMR (DMSO-d_6, δ ppm)**:7.69 (d,1H,Ar-H_7), 7.14 (d,1H,Ar-H_4),7.06 (dd,1H,Ar-H_6), 4.44(s,2H,CH_2COO), 4.12 (q,2H, CH_3-$\underline{CH_2}$-O), 3.84 (s,3H,OCH_3), 2.97 (q,4H,2(CH_3-$\underline{CH_2}$-N),1.13 (t,9H, 3(CH_3); Elemental analysis Calcd. for $C_{17}H_{25}N_3O_3S$: C,58.09; H,7.17; N,11.96; S,9.12. Found: C,58.07; H,6.83; N,11.35; S,9.48.

Synthesis of ethyl 2-(5-methoxy-2-(morpholinomethyl)thio)-1*H*-benzo[d] imidazole-1-yl)acetate (9)

White to off-white powder, yield: 70%; m.p. 146-149°C; **IR (υ=cm^{-1},KBr)**:3030 (υ Ar.-H) str, 2968 and 2872 (υ aliph. CH_3 a*sym.* and *sym.*)str, 2910 and 2818 (υ aliph. CH_2 a*sym.* and *sym.*)str, 1730(υ C=O ester) str, , 1631 (υ C=N) str, 1502 (υ Ar-C=C)str, 1261and 1018 (υ C-O methyl ether)str, 1197(υ C-O ester)str, 1155 (υ C-N)str, 623 (υ C-S)str; **^{1}HNMR (DMSO-d_6, δ ppm)**: 7.56 (d,1H, Ar-H_7), 7.13 (d,1H,Ar-H_4),7.03 (dd,1H, Ar-H_6), 4.39 (s,2H,CH_2-COO) , 4.61 (td,2H, OCH_2), 4.13 (q,2H, CH_3-**CH_2**-O),3.84 (s,3H,OCH_3), 3.77 (dd,2H,OCH_2) , 3.54 (d,2H, N-CH_2), 2.65 (td,2H,N-CH_2), 1.16 (t,3H,CH_3); Elemental analysis Calcd. for $C_{17}H_{23}N_3O_4S$: C,55.87; H,6.34; N,11.50; S,8.77. Found: C,55.80; H,6.43; N,10.96; S,9.07.

Synthesis of ethyl 2-(5-methoxy-2-(piperidine-1-ylmethyl)thio)-1*H*-benzo[d] imidazole -1-yl) acetate (10)

Off-white powder, yield: 65%; m.p. 135-137°C; **IR (υ=cm^{-1},KBr)**: 3078 (υ Ar-H), 2972 and 2880 (υ aliph. CH_3 a*sym.* and *sym.*)str, 2906 (υ aliph.CH_2 a*sym.*)str, (υ CH_2 *sym.* was overlapped with that of alph. CH_3)str, 1730 (υ C=O ester)str, 1635 (υ C=N)str, 1506 (υAr-C=C)str, 1271 and 1020 (υ C-O methyl ether)str, 1199 (υ C-O ester)str, 1159 (υ C-N)str, 623(υ C-S)str; **^{1}HNMR (DMSO-d_6, δ ppm)**:7.54 (d,1H,Ar-H_7), 7.11 (d,1H,Ar-H_4),7.02 (dd,1H, Ar-H_6), 4.36 (s,2H, CH_2-COO), 4.13 (q,2H, CH_3-**CH_2**-O), 3.83(s,3H,OCH_3), 2.68 (m,4H, piperidine), 1.56 (m,4H,piperidine), 1.25 (m,2H, piperidine), 1.16 (t,3H,CH_3); Elemental analysis Calcd.for $C_{18}H_{25}N_3O_3S$: C,59.48; H,6.93; N,11.56; S,8.82. Found: C,58.80; H,7.02; N,11.91; S,9.12.

2.4. Synthesis of 2-(5-methoxy-2-(piperidine-1-ylmethyl)thio)-1*H*-benzo[*d*] imidazole- 1-yl) acetamide (11) [11]

Compound **(10)** (0.022 mol, 8.10g,) dissolved in methanolic ammonia solution (33% w/v, 15ml) with stirring at room temperature overnight, the progress of the reaction was monitoring by TLC. The solvent evaporated and the residue was co-evaporated twice with chloroform (3 x25ml) ,collect the solid and recrystallized from hot methanol.

Off-white to beige powder, yield: 70%; m.p. 124-126°C; **IR (υ=cm^{-1},KBr)**: 3419 and 3296 (υ N-H *prim*. amine)str, 3078 (υ Ar-H)str, 2995 and 2899 (υ aliph. CH_3 a*sym.* and *sym.*)str, 2933 and 2831 (υ aliph. CH_2 a*sym.* and *sym.*)str, 1668(υ C=O amide)str, 1631(υ C=N)str, 1593(υ Ar-C=C)str, 1270 and 1028(υ C-O methyl ether)str,1163 (υ C-N)str, 626(υ C-S)str; **^{1}HNMR (DMSO-d_6, δ ppm)**:7.75(s,2H,NH_2-amide), 7.31 (d,1H,Ar-H_7), 7.24 (d,1H,Ar-H_4), 6.73 (dd,1H,Ar-H_6), 4.66 (s,2H,**CH_2**-CO-NH_2) , 3.98 (s,2H,CH_2-N-piperidine), 3.83 (s,3H,OCH_3), 2.68 (m,4H,piperidine), 1.66 (m, 4H,piperidine), 1.25 (m,2H,piperidine); Elemental analysis Calcd. for $C_{16}H_{22}N_4O_2S$:, C,57.46; H,6.63; N,16.75;S(9.59. Found: C,57.07; H,6.48; N,16.95; S,9.12.

2.5. General method for the synthesis of compounds (12 and 13) [12]

Appropriate primary or secondary amine (0.01 mol) (1.33g 2-amino BIM, 3.63 g of piperidine) and was gradually added to a solution of compound **(11)**, 0.01 mol, 3.34 g) in abs.methanol (12 ml), followed by the addition of an excess formaldehyde solution (38%,0.12mol,6.4ml). The reaction mixture was stirred for 1h at room temperature and allowed to stand overnight at 0ºC. Then the precipitate was filtered, dried and recrystallized using hot ethanol.

Column chromatography was run using S.G (60-120 mesh) and the mobile phase *n*-hexane: methanol: ethyl acetate (3.0:1.5:0.5) for **12** and *n*-hexane: ethyl acetate: ethanol (4.0:2.0:1.0) for **13** to purify the titled products.

Synthesis of 2-(5-methoxy-2-(piperidine-1-ylmethyl)thio)-1*H*-benzo[d]imidazole-1-yl)-N-(piperidine-1-ylmethyl)acetamide (12)

Beige powder, yield: 55%; m.p. 260-263°C; **IR (υ=cm^{-1},KBr)**: 3400(υ N-H amide)str, 3055(υ Ar-H)str, 2935 and 2852(υ aliph.CH_3 a*sym.* and *sym.*)str, 1674(υ C=O amide)str, 1614(υ C=N)str, 1568 (υ Ar-C=C)str, 1273and 1029 (υ C-O methyl ether)str, 1153 (υ C-N)str, 619 (υ C-S)str; **^{1}HNMR (DMSO-d_6, δ ppm)**: 7.86 (s,1H, HN-CO), 7.32 (d,1H,Ar-H_7), 6.96 (d,1H,Ar-H_4), 6.73 (dd,1H,Ar-H_6),3.97 (s,2H, (CH_2-N), 3.75 (s,3H,OCH_3), 2.68-1.24 (m,10H-piperidine); Elemental analysis Calcd. for $C_{22}H_{33}N_5O_2S$: C,61.22; H,7.71; N,16.23;S,7.43. Found: C,61.34; H,7.89; N,15.80; S,7.17.

Synthesis of N-((1*H*-benzo[d]imidazole-2-ylamino)methyl)-2-(5-methoxy-2-(piperidine -1-ylmethyl) thio) -1*H*-benzo[d]imidazole-1-yl)acetamide (13)

Off-white powder, yield: 70%; m.p. 99-102°C; **IR (υ=cm^{-1},KBr)**: 3377(υ N-H amide), (υ N-H *sec.* amine)was

overlapped with that of *sec*. amide, 3062 (υ Ar.-H), 2926 and 2852(υ aliph.CH_3 a*sym.* and *sym.*), 1678(υ C=O amide), 1620(υ C=N),1560 (Ar-C=C) str, 1273 and 1028 (υ C-O methyl ether)str, 1153(υ C-N)str, 617(υ C-S)str; **^{1}HNMR (DMSO-d_6, δ ppm)**: 7.79 (s,1H,NH-amide), 7.42-6.73 (d,7H,Ar-H), 4.20 (*br*s,1H,NH), 3.96 (s,2H,CH_2-N-BIM), 3.76 (s,3H,OCH_3), 2.64 (m,4H,piperidine) , 1.77 (m,4H,piperidine),1.24 (m,2H,piperidine); Elemental analysis Calcd.for $C_{24}H_{29}N_7O_2S$: C,60.10; H,6.09; N,20.44; S,6.69. Found: C,59.878; H,6.41; N,20.87; S,6.48.

2.6. Antimicrobial Sensitivity Assay:

Well, diffusion assay was carried out by using bacterial suspension of about (1.5×10^8 CFU/ml) obtained from McFarland turbidity standard (number 0.5).

This was used to inoculate by swabbing the surface of MHA plates. The excess liquid was air-dried under a sterile hood. In each agar plate of tested bacteria, five wells were made and (80µl) of each concentration was added to it.

The plates were incubated at 30 °C for 72 h (fungi spp.) or 37 °C for 24 h (bacteria), and the antimicrobial activity was evaluated by measuring the diameter of the inhibition zone (ZI) around the disc in mm.

Table 1: Physical data of the title compounds (1-13).

Comp. No.	m.p.°C	%Yield	*R_f	physical appearance	recrystallization solvent
1	125-128	93	0.85[c]	Off-white to beige powder	Ethanol
2	146-149	72	0.32[c]	Off-white to beige powder	Ethanol
3	60-61	76	0.65[e]	Beige powder.	Ethanol
4	79-82	76	0.35[b]	Off-white powder.	Ethanol
5	135-137	75	0.70[c]	Off-white powder	Ethanol
6	147-151	73	0.85[a]	Beige powder	Ethanol
7	148-150	65	0.90[a]	Pale-yellow powder	Ethanol
8	120-123	67	0.80[d]	Off-white powder	Chloroform
9	146-149	70	0.47[d]	White to off-white powder	Chloroform
10	135-137	65	0.57[a]	Off-white powder	Chloroform
11	124-126	70	0.63[b]	Off-white to beige powder	Methanol
12	260-263	55	0.70[f]	Beige powder	Ethanol
13	99-102	70	0.55[f]	Off-white powder	Ethanol

Note**: **The solvents ratio of each R_f corresponding to the synthesized compound.

a)n-hexane: ethyl acetate (6..0:4.0).*;b) n*-hexane: ethyl acetate (8.0:2.0); c). chloroform : acetone (9.5: 0.5); d) chloroform: acetone (8.0: 2.0); e) chloroform: CH_2Cl_2 (6.0:4.0); f*) n*-hexane: methanol: (7.0:3.0).

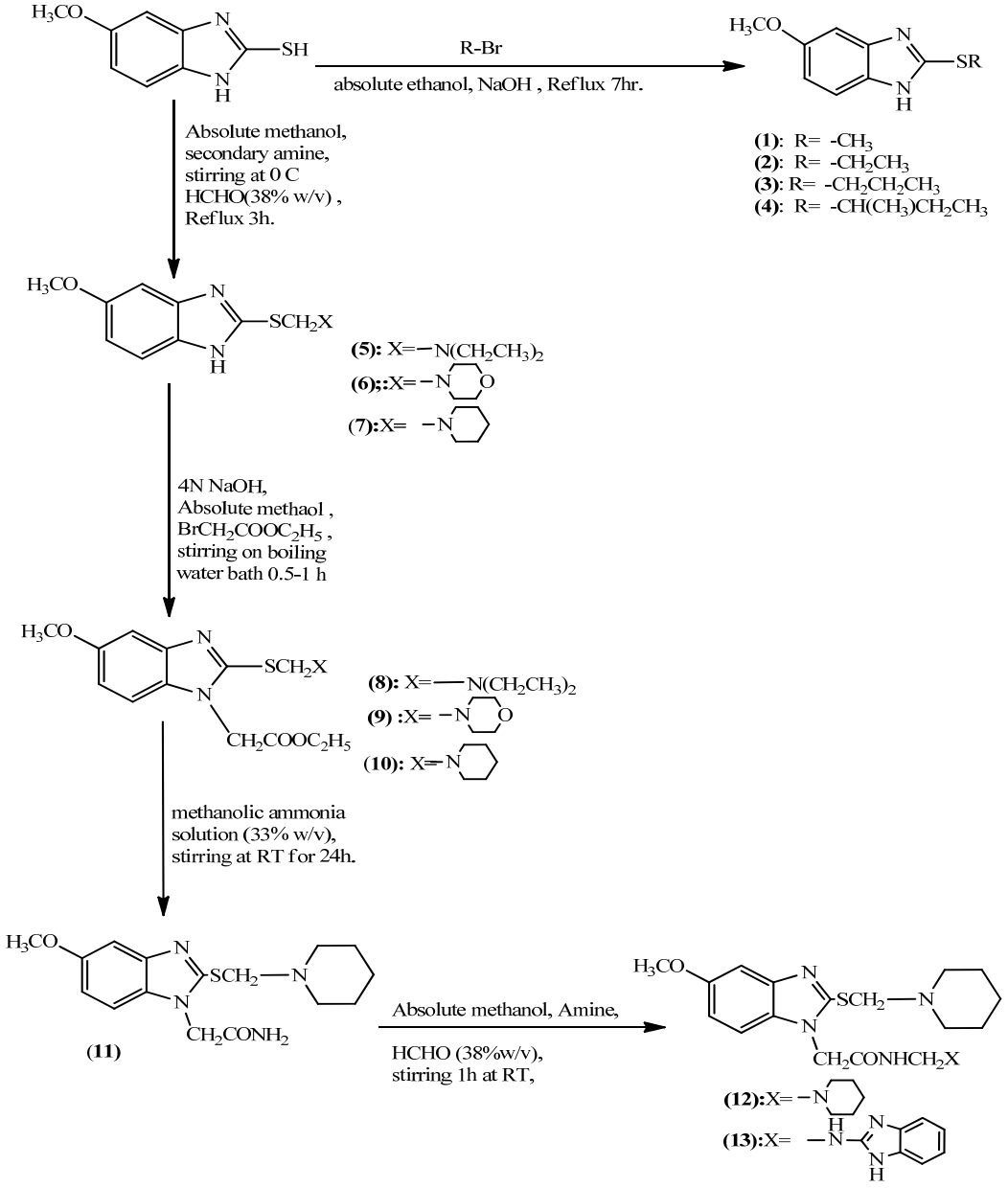
3.0 RESULTS AND DISCUSSION

3.1 *Chemistry*

The synthesis of compounds, **1-13** was accomplished and outlined in the **scheme 1**. The IR spectra of all compounds showed medium absorption bands at 700-600 cm^{-1}, (1275-1200) cm^{-1} and (1075-1020) cm^{-1} that accounted for (C-S) group and for *asymmetric* and *symmetric* aryl methyl ether respectively. The compounds **1-4** showed absorption bands, due to (NH) stretching at (3385-3153cm^{-1}), and bands at 2997, 2929 cm^{-1} due to (CH) *asymmetric* and *symmetric* (CH_3) stretching for the compound **1,** while compounds **2** and **3** showed characteristic bands at 2953, 2827 cm^{-1} and 2960, 2835 cm^{-1}, respectively, due to *asymmetric* and *symmetric* (CH_3) stretching. The compounds **5-7** displayed (NH)-stretching bands at the range (3390-3185) cm^{-1}, (CH) stretching of the aromatic ring at (3124-3109) cm^{-1}, and aliphatic (CH) stretching at (2962-2831) cm^{-1}. Moreover, the compounds **8-10** displayed characteristic bands at 1730 cm^{-1} due to (C=O) stretching of the ester, and characteristic bands at 1199,1197 and 1199 cm^{-1} respectively, due to (C-O) ester stretching, respectively. The compound **11** demonstrated an absorption band at 1668cm^{-1} due to (C=O) stretching of a primary amide, and characteristic absorption bands appeared at 3419, 3296cm^{-1} due to *asymmetric* and *symmetric* (NH_2) stretching of the primary amide. While the compounds (**12** and **13)** showed absorption bands at 3400 cm^{-1} accounted for (NH) stretching of *sec*. amide. Also, characteristic bands displayed at (1674and1678) cm^{-1} attributed to (C=O) stretching of the amide for the compounds **12** and **13** respectively. The ^{1}HNMR for the title compounds **1-13** displayed characteristic bands in the range δ = 7.56- 6.73 ppm attributed to the three benzimidazole aromatic protons, (**Ar-H_7, H_6,** and **H_4),** also a *singlet* prominent peak appeared at the δ = 3.84-3.76 pm due to the three protons of the methoxyl group (**OCH_3**) at the **C_5** of the molecule. Compound **1** showed a *singlet* peak at δ=2.67 and δ=2.69 ppm respectively due to (**CH_3-S**), while compound **2** displayed two signals of the alkyl side chain at δ= 2.69 ppm as a *quartet*, and δ=1.22 ppm as a *triplet*, due to **(CH_2)** and **(CH_3)** groups, respectively. The compound **3** illustrated the following peaks at δ= 3.21ppm as a *triplet*, δ=1.71ppm as a *multiplet* and δ=0.99ppm as a *triplet* accounted for the two methylene groups and the terminal three protons of the methyl group **(CH_3)**,

respectively. The **scheme 1**, illustrated the treatment of 5-methoxy-2-MBIM with formaldehyde and refluxing with appropriate *sec*. amines, (*S*-Mannich reaction), afforded the compounds **5-7.** The Compound **6** demonstrated the characteristic signals due to the eight protons of the morpholine ring at δ= 4.61-2.65 ppm. While the compound **7** displayed the ten protons of the piperidine ring at δ = 2.68-1.25 ppm, as a *multiplet* . On the other hand, the treatment of compounds **5-7** with ethyl bromoacetate in the presence of absolute methanol and stirring in the boiling water ,afforded the compounds **8-10**, and showed a characteristic peak at δ= 4.44-4.36 ppm as a *singlet* ,due to the two protons of the methylene group (**CH_2**-COO-) , and another important signal at δ= 4.13-4.12 ppm, *as a quartet*, due to the methylene group of the ester side chain(CH_3-**CH_2**-O-), while the three protons of the methyl group (**CH_3**-CH_2-O), appeared as a *triplet*, at δ =1.15,1.16 and 1.16 ppm for the compounds **8, 9,** and **10**, respectively.

The treatment of compound**10** with methanolic ammonia solution and stirring at RT, produced the compound **11 ,** with a broad *singlet* peak at δ =7.75 ppm, due to the two protons of (**NH_2**-amide), while a signal displayed at δ=4.66 ppm as a *singlet*, attributed to the two protons of methylene group (**CH_2**-CO), and other characteristic peak at δ= 3.98 ppm due to methylene group (S-**CH_2**-N-piperidine). The treatment of compound **11** with formaldehyde and each of appropriate amine (piperidine and 2-amino-BIM) with stirring at RT and then allowed to stand overnight at 0 °C, afforded the compounds **12** and **13**. Both of them showed distinct *singlet* peaks, at δ=7.86, δ=7.79 ppm, respectively, due to the proton of amide (**NH**-CO), other broad *singlet* peaks displayed at δ=3.97 and δ=3.96 ppm, respectively, attributed to the two protons of the methylene group, (NH-**CH_2**-amine).



Scheme 1: Synthesis of title compounds (1-13)

3.2 *Antimicrobial evaluation*:

The antimicrobial activities of the synthesized derivatives of 5-methoxy-2-mercapto benzimidazole were measured using well diffusion technique**[13]** with a comparison to cefotaxime sodium, sulphamethoxazole, and miconazole as standard antibacterial and antifungal agents, by using DMF as solvent and control, as shown in the following tables**, 2** and **3** respectively.

Table 2: The antibacterial activity of the synthesized compounds (1-13)

Comp. No.	Conc. µg/ml	*S. aureus* ATCC: 6538	*Bacillus subtilis* ATCC: 6051	*Bacillus pumilus* NCTC: 8241	*Pseud. aeruginosa* ATCC: 9027	*E. coli* ATCC: 8739	*Enterobacter cloacae* ATCC: 13047
		Inhibition zone in (mm)					
1	50	-	-	-	-	**8.9**	**11**
	100	-	-	-	-	**9.2**	**15**
2	50	-	-	-	-	**8.8**	**14**
	100	-	-	-	-	**10.6**	**15**
3	50	-	-	-	-	**9.0**	**14**
	100	-	-	-	-	**10.0**	**15**
4	50	-	-	-	-	**8.3**	**12**
	100	-	-	-	-	**11.4**	**15**
5	50	-	-	-	-	**7.7**	**12**
	100	-	-	-	-	**10.08**	**12**
6	50	-	-	-	-	**8.6**	**11**
	100	-	-	-	-	**11.0**	**12**
7	50	-	-	-	-	**7.6**	**13**
	100	-	-	-	-	**10.01**	**13**
8	50	-	-	-	-	**8.7**	**11**
	100	-	-	-	-	**10.53**	**13**
9	50	-	-	-	-	**7.6**	**12**
	100	-	-	-	-	**10.3**	**12**
10	50	-	-	-	-	**7.8**	**11**
	100	-	-	-	-	**10.05**	**14**
11	50	-	-	-	-	**9**	**12**
	100	-	-	-	-	**10.57**	**13**
12	50	-	-	-	-	**8.8**	**13**
	100	-	-	-	-	**10.36**	**13**
13	50	-	-	-	-	**8.1**	**12**
	100	-	-	-	-	**10.99**	**14**
Cefot.	***50***	-	***25***	***20***	***18***	***11***	-
	100	***15***	***30***	***25***	***22***	***13***	***15***
Sulf.	***50***	-	***15***	***18***	***13***	***9.1***	***14***
	100	-	***20***	***22***	***18***	***14.1***	***14***
DMF	-	-	-	-	-	-	-

Cefot.;(Cefotaxime sodium), sulph.;(sulfamethoxazole), (-)= No activity, (+)= slightly active (Inhibition Zone in between 5-10 mm), (++)= moderately active (Inhibition zone , between 10-15 mm), (+++)= highly active (Inhibition zone more than 15 mm).

Table 3: The antifungal activity of the synthesized compounds (1-13)

Comp. No.	Conc. µg/ml	*Aspergillus Niger* ATCC:1015	*Penicillium expansum* ATCC: 7861	*Candida albicans* ATCC: 10231
		Inhibition zone (mm)		
1	**100**	-	-	-
2	**100**	-	-	-
3	**100**	-	-	-
4	**100**	-	-	-
5	**100**	-	-	-
6	**100**	-	-	-
7	**100**	-	-	-
8	**100**	-	-	-
9	**100**	-	-	-
10	**100**	-	-	-
11	**100**	-	-	-
12	**100**	**9**	-	-
13	**100**	**10**	-	-
Miconazole	***100***	***8***	***10***	***22***
DMF	-	-	-	-

(-)= No activity, (+) = slightly active (Inhibition zone between 5-10 mm), (++) = moderately active (Inhibition zone between 10-15 mm), (+++)= highly active (Inhibition zone more than 15 mm).

The recorded data, tables **2**and **3** lead to the following conclusion:

All the tested compounds exhibited moderate to strong activity against Gram- negative bacteria (*except for pseudomonas aeruginosa,* gives no activity), in which, the compound **4,** and **6** showed the greatest antibacterial activity against Gram-negative *E.coli* at a concentration of 100µg/ml.

On the other hand, the compounds **(1-4**) and **(10** and **13)** showed the greatest antibacterial activity against Gram-negative *Enterobacter cloacae* at a concentration of 100µg/ml.

All the synthesized compounds showed no antibacterial activity against Gram-positive used bacteria.

It is evident that only the compounds **12** and **13** demonstrated the highest antifungal activity against *Aspergillus niger,* at a concentration of 100 µg/ml.

CONCLUSION

A new series of 5-methoxy-2-mercapto benzimidazole derivatives were successfully synthesized and tested for their antimicrobial activities.

S-alkylation of the synthesized ring displayed remarkable antibacterial activities against Gram-negative types especially compounds **1-4,** showed the maximum activity against *Enterobacter cloacae*, while other derivatives demonstrated various antibacterial activities against Gram-negative bacteria, and some fungal species, especially compounds **12** and **13**, exhibited highest antifungal activity against *Aspergillus niger.*

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CONFLICT OF INTEREST:

The authors declare no conflict of interest

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