Raad H. Turkey et al. / Journal of Pharmacy Research 2016,10(12),814-824

Research Article
ISSN: 0974-6943

Available online through
http://jprsolutions.info

Synthesis, characterization and antibacterial activity of new 5-ethoxy-2-mercapto benzimidazole derivatives

Raad H. Turkey[1] and Ammar Abdul Razzak M. Kubba[2,*]

[1]*Ministry of health-Karbala Health Directorate, Pharmacy Department*
[2]*College of Pharmacy-Dept. of Pharmaceutical Chemistry, Bab-Almuadam,Baghdad-Iraq.*

Received on:22-09-2016; Revised on: 27-10-2016; Accepted on: 16-12-2016

ABSTRACT

Background: In this study, new derivatives were synthesized from 5-ethoxy-2-mercapto benzimidazole by alkylation, acylation, Schiff base formation and vilsmier-haack reaction (ring closure), and in a good yield. **Methods:** The reaction of 5-ethoxy-2-mercapto benzimidazole with different types of alkyl halides to produce S-alkyl derivatives (thioether),**1a-c**. The reflux of the parent compound with ethyl bromoacetate, afforded the thioester form of 5-ethoxy2-mercapto benzimidazole derivative, and by treating with benzoyl chloride, lead to the formation of the N-benzoyl thioester form **2**. The formation of the thiazolo ring-containing compound **3** was done by equimolar reflux of aliphatic ketone and 5-ethoxy-2-mercapto benzimidazole.The compounds **4a-d** were prepared by the reaction of the parent compound with *p*-substituted phenacyl bromides, then by addition of 2,4-dinitrophenyl-hydrazine produced the Schiff bases **5a-d**. The pyrazole derivatives **6a-d** produced by using vilsmier-Haack reaction, by the treatment of **5a-d** with phosphorous oxychloride and DMF. New series of compounds **7a-d** and **8** were obtained by the reaction of **4a-d** with benzoyl chloride and acetic anhydride, respectively. The antimicrobial activity was evaluated by using Agar Diffusion Well Assay. The four types of bacteria used to evaluate the *in-vitro* antibacterial activity were *Staphylococcus aureus*, *Streptococcus agalactiae*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. All the titled compounds characterized and identified by elemental microanalysis, I.R, and ^{1}HNMR spectroscopic study. It was found that all the results showed good agreements with the proposed chemical structures of the synthesized compounds. **Results:** All the new compounds have distinctive and highest antibacterial activity against Gram-positive bacteria, especially **6b** showed the maximum antibacterial activity against *Staph. aureus* at a concentration of 100µg/ml, while the compound **7b** exhibited the maximum antibacterial activities against both Gram-positive and Gram–negative bacteria at a concentration of 100µg/ml. **Conclusion:** The results illustrated good preliminary antibacterial activity of the new 5-ethoxy-2-mercapto benzimidazole derivatives, especially **6b** and **7b**, against Gram-positive and Gram-negative bacteria.

KEYWORDS: Benzimidazole derivatives, 5-ethoxy-2-mercapto benzimidazole, Schiff base, vilsmier-haack reaction

1. INTRODUCTION

Heterocyclic compounds are acquiring more importance in recent years due to the pharmacological activities[1].They have important applications in organic synthesis as organocatalysts, synthetic intermediates, chiral auxiliaries, and metal ligands in asymmetric catalysis. Therefore, the development of new efficient methods to synthesize heterocycles is of considerable interest[2]. Benzimidazole is a heterocyclic aromatic organic compound. It is an important pharmacophore and a privileged structure in medicinal chemistry[3]. Benzimidazoles possess different biological activities such as anti-inflammatory, diuretic, antimicrobial, antibacterial, antiviral, antitumor, antiprotozoal ,antiulcer, protein kinase CK2, antioxidants, antiasthmatic, antidiabetic, 5-HT3 receptor antagonist, analgesic, hypotensive, anti-mycobacterial, anthelmintic, histamine H4 receptor antagonist, and anticonvulsant activity[4],[5]. 2-Mercapto benzimidazole derivatives are one of the most important derivatives of benzimidazole known to possess varied biological activities, such as antihistamine [6], anti-diabetic[7], hypocholesterolemic activity[8], anxiolytic[9], anti-cancer [10], anti-convulsant [11], analgesic [12], anti–inflammatory [13], actoprotector [14],anti-ulcer[15], antifungal [16], antibacterial [16],and antiprotozoal[17].The aim of the present study is to design, synthesize and evaluate *in vitro* the antibacterial activity of new derivatives of 5-ethoxy-2-mercapto benzimidazole, which were synthesized by S-alkylation with different alkyl halides, S-acylation, and N-acylation with derivatives of *p*-substituted phenacyl bromides, and then, the formation of various Schiff bases through the reaction with 2,4-dinitro-phenylhydrazine.

***Corresponding author.**
Dr. Ammar A.Razzak Mahmood Kubba
College of Pharmacy-Dept. of Pharmaceutical Chemistry,
Bab-Almuadam,Baghdad-IRAQ

2. MATERIALS AND METHODS

The completion of reaction and the purity of the products was monitored by thin-layer chromatography (TLC) using silica gel GF_{254}

(type 60) pre-coated Aluminum sheets, Merck (Germany), the spots of the final compounds were visualized by irradiation with UV light or by using reaction with iodine vapor or using an original Shimadzu UV-light spectrometry. The chemicals and solvents were purchased from Hangzhou Hyper Chemicals Limited Company, ROMIL, and Himedia companies. Melting points of the synthesized compounds were determined in open capillary tubes and were uncorrected, and by using the apparatus Stuart Electronic Melting Point. Determinations of infrared spectra were recorded in KBr disc, ($\upsilon = cm^{-1}$) using FTIR spectrophotometer, The proton ^{1}HNMR spectra were recorded on (Bruker, Germany NMR Spectrometer 400 MHz, Avance III 400 spectrometer) in Central Laboratory Isfahan University-Iran, the chemical shifts are reported in δ values (part per million) relative to tetramethylsilane (TMS) as an internal standard. The elemental microanalysis of the synthesized final derivatives was recorded on microanalyzer (EuroEA 3000, Europe) in the University of Baghdad-College of Education for Pure Science, Ibn Al-Haitham Advisory Office the Central Service Laboratory.The results of the elemental analysis (CHN) were found to be in good agreement (-+ 0.5%) with the calculated values. 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 of (1a-c) [18].

(3.88 g, 0.02 mol) of 5-ethoxy-2-MBI was dissolved in absolute ethanol (15 ml) with an alkyl halide (0.02mol) (methyl bromide1.89 g, isopropyl bromide 2.46 g, benzyl bromide 2.53 g), and sodium hydroxide (0.8 g, 0.02 mol) in a round flask (50 ml) and reflux condenser. The mixture was refluxed for 4-7 h according to TLC, and filtered directly to get rid of the precipitated salt. The filtered sample cooled and recrystallized from ethanol.

Synthesis of 5-ethoxy-2-(methylthio)-1H-benzo[d]imidazole(1a):

Light brown powder, Yield 76%; m. p. 94-96 ˚C; **IR (υ= cm^{-1},KBr):** 3101 Ar(CH) str., 2970 (sym. CH_3) str., 2881 (sym. CH_3) str., 2926 (asym. CH_2) str., 2821 (sym. CH_2) str., 1629 (C=N) str. 1597 Ar (C=C) str.; 1273 (asym. C-O-C) str., 1045 (sym. C-O-C str.), 673 (C-S) str **;** **^{1}HNMR (400MHz), DMSO-d_6,δ ppm):** 7.31(d,1H,Ar-H_7), 6.94 (s,1H, Ar-H_4), 6.72(dd, 1H, Ar-H_6), 4.01(q, 2H,CH_2), 2.66(s,3H,S-CH_3), 1.33 (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, 58.005; H, 5.628; N, 13.767; S, 15.00.

Synthesis of 5-ethoxy-2-(isopropylthio)-1H-benzo[d]imidazole(1b):

Beige powder, Yield 61%; m. p. 124-126 °C; **IR (υ= cm^{-1},KBr):** 3037 Ar (CH) str., 2974 (asym. CH_3) str., 2866 (sym. CH_3) str., 2928 (asym. CH_2) str., 1626 (C=N) str., 1593Ar(C=C) str., 1396 (CH(CH_3)$_2$) gem dimethyl bend., 1271 (asym. C-O-C) str., 1047 (sym. C-O-C) str., 673 (C-S) str**.;** **^{1}HNMR (400MHz),DMSO-d_6,δ ppm):** 7.33(d,1H,Ar-H_7), 6.95 (d,1H,Ar-H_4), 6.72 (dd,1H,Ar-H_6), 4.03(q,2H, CH_2), 3.88(m,1H,CH (CH_3)$_2$), 1.35(d,6H, 2CH_3), 1.32(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, 61.005; H, 6.695; N, 11.183; S, 13.39.

Synthesis of 2-(benzylthio)-5-ethoxy-1H-benzo[d]imidazole (1c):

Brown crystals, Yield 91%; m. p. 89-91 °C; **IR (υ= cm^{-1},KBr):** 3059 Ar(CH) str., 2980 (asym. CH_3) str., 2870 (sym. CH_3) str, 2931 (asym. CH_2) str., 1629 (C=N) str., 1589 Ar(C=C) str., 1274 (asym. C-O-C) str., 1039 (sym.C-O-C) str.,671 (C-S) str**.;** **^{1}HNMR (400MHz),DMSO-d_6, δ ppm):** 7.43 (d,1H,Ar-H_7), 6.95(d,1H, Ar-H_4), 6.75(dd, 1H, Ar-H_6),7.33-7.23 (m,5H, Ar-H_5), 4.53 (s,2H, S-CH_2), 4.02(q, 2H, CH_2), 1.34 (t,3H, CH_3); Elemental analysis Calcd. for $C_{16}H_{16}N_2OS$: C, 66.64; H, 5.22; N, 10.36; S, 11.86. Found: C, 66.341; H, 5.764; N, 10.774; S, 11.939**.**

2.2. Synthesis of ethyl 2-[(1-benzoyl-5-ethoxy-1H-benzo[d]imidazole-2-yl]thio)acetate (2) [19].

Step 1: 5-ethoxy-2-mercapto benzimidazole (4.85 g , 0.025mol) and ethyl bromoacetate (2.8 ml,0.025mol) in dry acetone (20 ml) in the presence of K_2CO_3 (3.45g ,0.025mol) was refluxed for 5h and the reaction mixture poured into ice water, and neutralized with dil. HCl, the solid thus, obtained was washed several times with water and recrystallized from ethanol.

Step 2: An equimolar mixture of the thioester derivative from step one,(2.8g,0.01mol) and benzoyl chloride(1.16ml,0.01mol) in aqueous NaOH(10%)solution was stirred for 10-12 h at R.T.,A solid precipitate that separated was filtered off and washed with dil. HCl, recrystallized from ethanol and finally produced a light beige powder. Yield 74%; m.p. 161-163 °C; **IR (υ= cm^{-1},KBr):** 3053 Ar(CH) str., 2978 (asym. CH_3) str., 2879 (sym. CH_3) str, 2929 (asym. CH_2) str., 2820 (sym. CH_2) str., 1710 (C=O) ester str. 1637 (C=N) str., 1581 Ar(C=C) str., 1273 (asym. C-O-C) str., 1041 (sym. C-O-C) str., 682 (C-S) str.**;** **^{1}HNMR (400MHz),DMSO-d_6,δ ppm):** 7.49-7.46 (m,5H, Ar-H), 7.31(d,1H,Ar-H_7), 6.95 (d,1H,Ar-H_4), 6.73 (dd,1H,Ar-H_6), 4.09 (s,2H,S-CH_2), 4.03 (q,4H, 2CH_2),1.33(t,6H, 2CH_3); Elemental analysis Calcd. for $C_{20}H_{22}N_2O_4S$: C, 62.16; H, 5.74; N, 7.25; S, 8.30. Found: C, 62.991; H, 5.488; N, 7.558; S, 8.809.

2.3. Synthesis of 6-ethoxy-2-isopropyl-3-methylbenzo [4,5]imidazo[2,1-b]thiazole (3) [20].

A mixture of 5-ethoxy-2-mercapto benzimidazole (0.97 g, 0.005mol) and 4-methyl-2-pentanone (0.77 ml, 0.005mol) was refluxed in acetic acid (20 ml) containing a few drops of conc. H_2SO_4 as a catalyst for 4h. The reaction mixture left with stirring at room temperature

overnight. The reaction mixture was diluted with NH_4OH, washed with water, extracted with dichloromethane (3x20 ml) and the combined extract was dried ($MgSO_4$).The dichloromethane was removed from the filtrate and the resulting black powder was recrystallized from dilute ethanol to give the product (**3**).

Yield 86%; m. p. 240-242 °C; **IR (υ= cm^{-1}, KBr):** 3088, 3064 Ar(CH) str. 2976 (asym. CH_3) str., 2875 (sym. CH_3) str., 2897 (asym. CH_2) str., 2841 (sym. CH_2) str., 1635 (C=N) str., 1498 Ar(C=C) str., 1379, 1363 ($CH(CH_3)_2$) gem.dimethyl bend., 1259 (asym. C-O-C) str., 1047 (sym. C-O-C) str., 661 (C-S) str.; **^{1}HNMR (400MHz),DMSO-d$_6$,δ ppm):** 7.02(d,1H, J=8H$_Z$,Ar-H), 6.71(d,1H, J=8Hz,Ar-H), 6.66 (d,1H,Ar-H), 3.98 (q,2H, CH_2), 3.41(m,1H, $CH(CH_3)_2$), 2.47 (s,3H, CH_3), 1.31(t, 3H, CH_3), 1.21(d, 6H, $2CH_3$); elemental analysis Calcd. for $C_{15}H_{18}N_2OS$: C, 65.66; H, 6.61; N, 10.21; S, 11.96. Found: C, 65.424; H, 6.083; N, 10.495; S, 11.196.

2.4. General method for the synthesis of-1-(4-substituted phenyl)-2-((5-ethoxy-1H-benzo[d]imidazole-2-yl)thio)ethanone (4a-d) [21].

A mixture of 5-ethoxy-2-mercapto benzimidazole (0.48g, 0.0025 mol), *p*-substituted phenacyl bromide (0.0025mol), (phenacyl bromide 0.49 g , *p*-chloro phenacyl bromide 0.58 g, *p*-methoxy phenacyl bromide 0.57 g, *p*-nitro phenacyl bromide 0.61 g) and anhydrous K_2CO_3 (0.3g, 0.0025 mol) in dry acetone (30 ml) was refluxed for 2-3h. The filtrate was concentrated to dryness, the residue was treated with water, and the solid formed was filtered, washed with water, dried and recrystallized from acetone.

Synthesis of 2-[(5-ethoxy-1H-benzo[d]imidazol-2-yl)thio]-1-phenylethanone (4a):

Gray powder. Yield 94%; m. p. 203-205 °C; **IR (υ= cm^{-1},KBr):** 2978 (asym. CH_3) str., 2883 (sym. CH_3) str., 2924 (asym. CH_2) str., 1672 (C=O) str., 1647 (C=N) str., 1618, 1518, 1556 Ar(C=C) str., 1259 (asym. C-O-C) str.; 1047 (sym. C-O-C) str.; 653, 623 (C-S) str.; **^{1}HNMR (400MHz),DMSO-d$_6$,δ ppm):** 8.07-6.83(m,8H,Ar-H),5.70 (s, 2H, CH_2-S), 4.08 (q, 2H, CH_2), 1.33(t, 3H, CH_3); Elemental analysis Calcd. for $C_{17}H_{16}N_2O_2S$: C, 65.36; H, 5.16; N, 8.97; S, 10.26. Found: C, 65.334; H, 5.235; N, 8.498; S, 10.609.

Synthesis of 1-(4-chlorophenyl)-2-[(5-ethoxy-1H-benzo[d]imidazole-2-yl]thio)ethanone (4b):

Gray powder. Yield 92%; m. p. 231-233 °C; **IR (υ= cm^{-1},KBr):** 3014 Ar(CH) str., 2978 (asym. CH_3) str., 2831 (sym.CH_3) str., 2928 (asym. CH_2) str.,1674 (C=O) str., 1633 (C=N) str.,1587, 1570 Ar(C=C) str., 1259 (asym. C-O-C) str., 1045 (sym. C-O-C) str., 653, 624 (C-S) str.,576 (C-Cl) str.; **^{1}HNMR (400MHz),DMSO-d$_6$,δ ppm):** 8.09 (d,2H,Ar-H),7.70 (d,2H, Ar-H),7.48 (d,1H,Ar-H$_7$), 7.06 (d, 1H,Ar-H$_4$), 6.96 (dd,1H,Ar-H$_6$), 5.18 (s,2H,S-CH_2), 4.07 (q,2H,CH_2),1.35 (t,3H,CH_3); Elemental analysis Calcd. for $C_{17}H_{15}ClN_2O_2S$: C, 58.87; H, 4.36; N, 8.08; S, 9.25. Found: C, 58.925; H, 4.166; N, 8.769; S, 9.219.

Synthesis of 2-[(5-ethoxy-1H-benzo[d]imidazole-2-yl)thio]-1-(4-methoxyphenyl)ethanone(4c):

Blue powder. Yield 89%; m. p. 205-207 °C; **IR (υ= cm^{-1},KBr):** 2974(asym. CH_3) str., 2941 (sym. CH_2) str., 1660 (C=O) str., 1637 (C=N) str.,1600, 1573, 1543 Ar(C=C) str., 1217 (asym. C-O-C) str. for methoxy gr.,1028 (sym. C-O-C) str. for methoxy gr., 601 (C-S) str.; **^{1}HNMR (400MHz), DMSO-d$_6$,δ ppm):** 8.04 (d,2H,Ar-H),7.49 (d,2HAr-H),7.14 (d,1H,Ar-H$_7$),7.06 (d,1H,Ar-H$_4$), 6.96 (dd,1H,Ar-H$_6$), 5.18 (s,2H,S-CH_2), 4.08 (q,2H,CH_2), 3.88 (s,3H,OCH_3), 1.36 (t,3H,CH_3); Elemental analysis Calcd. for $C_{18}H_{18}N_2O_3S$: C, 63.14; H, 5.30; N, 8.18; S, 9.36. Found: C, 63.342; H, 5.688; N, 8.693; S, 9.423.

Synthesis of 2-[(5-ethoxy-1H-benzo[d]imidazole-2-yl)thio]-1-(4-nitrophenyl)ethanone (4d):

Beige powder. Yield 96%; m. p. 246-248 °C; **IR (υ= cm^{-1}, KBr):** 3009 Ar(CH) str., 2953 (asym. CH_3) str., 2881 (sym. CH_3) str., 2833 (sym. CH_2) str., 1695 (C=O) str., 1633 (C=N) str., 1600, 1556 Ar(C=C) str., 1230 (asym. C-O-C) str., 1043 (sym. C-O-C) str. 653, 623 (C-S) str., 1527 (asym.NO_2) str., 1346 (sym. NO_2) str ; **^{1}HNMR (400MHz), DMSO-d$_6$,δ ppm):** 8.43 (d,2H,Ar-H), 8.33 (d,2H, Ar-H), 7.03 (d,1H,Ar-H$_7$), 6.93 (d,1H,Ar-H$_4$), 6.72 (dd,1H, Ar-H$_6$), 5.26 (s,2H,S-CH_2), 4.07 (q,2H,CH_2), 1.33 (t,3H,CH_3); Elemental analysis calcd. for $C_{17}H_{15}N_3O_4S$: C, 57.13; H, 4.23; N, 11.76; S, 8.97. Found: C, 57.367; H, 4.655; N, 11.301; S, 8.130.

2.5. General metod for the Synthesis of 2-[(2-(4-substituted phenyl)-2-(2-(2,4-dinitrophenyl)hydrazono)ethyl)thio]-5-ethoxy-1H-benzo[d]imidazole (5a-d) [22].

A mixture of (**4a-d**), (0.005mol) (4a:1.56g, 4b:1.73g, 4c:1.71g, 4d:1.78g), 2,4-dinitro phenylhydrazine (0.992g, 0.005mol), methanol (20 ml) and conc. H_2SO_4 (0.4 ml) was stirred at room temperature for 3 h. After the completion of the reaction, the mixture poured into ice-cold water (50 ml). The separated solid filtered, washed with water (5×10 ml) and dried to obtain the crude product, which on recrystallization from ethanol afforded pure (**5a-d).**

Synthesis of 2-[(2-(2-(2,4-dinitrophenyl)hydrazono)-2-phenylethyl) thio]-5-ethoxy-1H-benzo[d]imidazole (5a):

Bright green powder, Yield 85%; m. p. 221-223 °C; **IR (υ= cm^{-1}, KBr):** 3090, 3064 Ar(CH) str., 2976 (asym. CH_3) str. 2848 (sym. CH_2) str., 1616

(C=N) str., 1593 (aromatic C=C) str., 1265 (asym. C-O-C) str., 1020 (sym. C-O-C) str., 661, 640 (C-S) str., 1504 (asym. NO_2) str., 1342 (sym. NO_2) str; **^{1}HNMR (400MHz), DMSO-d_6,δ ppm):** 11.05 (s,1H,NH), 8.42-7.46 (m,,11H, Ar-H), 4.78 (s,2H, CH_2-S), 4.02 (q,2H,CH_2), 1.31 (t,3H,CH_3); Elemental analysis Calcd. for $C_{23}H_{20}N_6O_5S$: C, 56.09; H, 4.09; N, 17.06; S, 6.51. Found: C, 55.955; H, 4.005; N, 16.526; S, 6.68.

Synthesis of 2-[(2-(4-chlorophenyl)-2-(2-(2,4-dinitrophenyl) hydrazono)ethyl)thio]-5-ethoxy-1H-benzo[d]imidazole (5b):

Bright green powder, Yield 79%; m. p. 198-200 °C; **IR (υ= cm^{-1}, KBr):** 3045 Ar(CH) str., 2978 (asym. CH_3) str., 2879 (sym. CH_3) str., 2933 (asym. CH_2) str., 2839 (sym. CH_2) str., 1616 (C=N) str., 1595 Ar(C=C) str., 1267 (asym. C-O-C) str., 1043 (sym. C-O-C) str., 653 (C-S) str., 1502 (asym. NO_2) str., 1344 (sym. NO_2) str., 576 (C-Cl) str; **^{1}HNMR (400MHz),DMSO-d_6,δ ppm):** 10.96 (s, 1H,NH), 8.95-6.71 (m, 10H, Ar-H), 4.76 (s,2H,CH_2-S), 3.98 (q,2H,CH_2), 1.32 (t,3H,CH_3); Elemental analysis . Calcd. for $C_{23}H_{19}ClN_6O_5S$: C, 52.42; H, 3.63; N, 15.95; S, 6.08. Found: C, 51.215; H, 3.575; N, 15.005; S, 6.142.

Synthesis of 2-[(2-(2-(2,4-dinitrophenyl)hydrazono)-2-(4-methoxyphenyl)ethyl)thio]-5-ethoxy-1H-benzo[d]imidazole (5c):

Yellowish green powder, Yield 83%; m. p. 188-190 °C; **IR (υ= cm^{-1}, KBr):** 3099, 3066 Ar(CH) str., 2980 (asym. CH_3) str., 2841 (sym. CH_2) str., 1616 (C=N) str., 1593 Ar(C=C) str.,1265 (asym. C-O-C) str. for ethoxy, 1066 (sym. C-O-C) str. for ethoxy gr., 1226 (asym. C-O-C) str.for methoxy gr., 1024 (sym. C-O-C) str. for methoxy gr., 607 (C-S) str., 1512 (asym. NO_2) str., 1340(sym. NO_2) str; **^{1}HNMR (400MHz),DMSO-d_6,δ ppm):** 11.16 (s,1H,NH), 8.96-7.02 (m,10H,Ar-H), 4.74 (s, 2H, CH_2-S), 3.98 (q,2H,CH_2), 3.88 (s,3H,OCH_3), 1.31 (t,3H,CH_3); Elemental analysis Calcd. for $C_{24}H_{22}N_6O_6S$: C, 55.17; H, 4.24; N, 16.08; S, 6.14. Found: C, 55.624; H, 4.126; N, 15.999; S, 6.004.

Synthesis of 2-[(2-(2-(2,4-dinitrophenyl)hydrazono)-2-(4-nitrophenyl)ethyl)thio]-5-ethoxy-1H-benzo[d]imidazole (5d):

Bright gray powder, Yield 90%; m. p. 240-242 °C; **IR (υ = cm^{-1}, KBr):** 3105 Ar(CH) str., 2937 (asym. CH_2) str., 1614 (C=N) str., 1598 Ar(C=C) str., 1265 (asym. C-O-C) str., 1020 (sym. C-O-C) str., 655 (C-S) str., 1518 (asym. NO_2) str.,1346 (sym. NO_2) str; **^{1}HNMR (400MHz),DMSO-d_6, δ ppm):** 10.82 (s,1H,NH), 8.99-7.86 (m,10H, Ar-H), 4.83(s,2H,CH_2-S), 3.98 (q, 2H, CH_2), 1.32 (t, 3H,CH_3); Elemental analysis Calcd. for $C_{23}H_{19}N_7O_7S$: C, 51.39; H, 3.56; N, 18.24; S, 5.97. Found: C, 51.855; H, 3.387; N, 18.953; S, 5.852.

2.6.General method for the synthesis of N-(2,4-dinitrophenyl)-4-[(5-ethoxy-1H-benzo[d]imidazole-2-yl)thio]-3-(4-substituted phenyl)-1H-pyrazole-1-amine (6a-d) [22].

A mixture of (**5a-d**), (0.005mol) (5a:2.47g, 5b:2.63g, 5c:2.61g, 5d:2.68g) and DMF (5 ml) allowed cooling in an ice bath with stirring. To the stirred solution, phosphorus oxychloride (0.8 ml, 0.0085mol) added drop- wise and the mixture stirred at room temperature for 4 h. At the end of this period, the mixture was poured into ice-cold water (50 ml).The separated solid was filtered, washed with water (3×10 ml) and dried to obtain the crude product which on recrystallization from ethyl acetate gave pure (**6a-d**).

Synthesis of N-(2,4-dinitrophenyl)-4-[(5-ethoxy-1H-benzo[d] imidazole-2-yl)thio]-3-phenyl-1H-pyrazole-1-amine (6a):

Yellow powder, Yield 60%; m. p. 105-107 °C; **IR (υ= cm^{-1}, KBr):** 3091 Ar(CH) str., 2978 (asym. CH_3) str., 1614 (C=N) str., 1593, 1502 Ar(C=C) str., 1269 (asym. C-O-C) str., 1047 (sym. C-O-C) str., 698, 628 (C-S) str., 1537 (asym. NO_2) str., 1338 (sym. NO_2) str; **^{1}HNMR (400MHz), DMSO-d_6 ,δ ppm):** 8.96-7.51(m,11H, Ar-H),6.66 (s, 1H, CH=C-), 4.06(q, 2H, CH_2), 1.33(t,3H,CH_3);Elemental analysis Calcd. for $C_{24}H_{18}N_6O_5S$: C, 57.36; H, 3.61; N, 16.72; S, 6.38. Found: C, 57.009; H, 3.030; N, 16.497; S, 6.311.

Synthesis of 3-(4-chlorophenyl)-N-(2,4-dinitrophenyl)-4-[(5-ethoxy-1H-benzo[d]imidazole-2-yl)thio]-1H-pyrazole-1-amine (6b):

Yellowish green powder, Yield 58%; m. p. 108-109 °C; **IR (υ= cm^{-1}, KBr):** 3093 Ar(CH) str., 2981(asym. CH_3) str., 1608 (C=N) str., 1494 Ar(C=C) str., 1271 (asym. C-O-C) str., 1041 (sym. C-O-C) str., 640 (C-S) str., 1537 (asym. NO_2) str., 1338 (sym. NO_2) str; **^{1}HNMR (400MHz),DMSO-d_6,δ ppm):** 8.97-7.84 (m, 10H, Ar-H), 6.70 (s,1H,CH=C-),4.02(q,2H,CH_2), 1.33(t, 3H,CH_3);Elemental analysis Calcd.for $C_{24}H_{17}ClN_6O_5S$: C, 53.68; H, 3.19; N, 15.78; S, 5.97. Found: C, 53.030; H, 3.682; N, 15.388; S, 5.995.

Synthesis of N-(2,4-dinitrophenyl)-4-[(5-ethoxy-1H-benzo[d]imidazole-2-yl)thio]-3-(4-methoxyphenyl)-1H-pyrazole-1-amine (6c):

Bright green powder, Yield 49%; m. p. 116-118 °C; **IR (υ= cm^{-1}, KBr):** 2978 (asym. CH_3) str., 2929 (asym. CH_2) str., 2837 (sym. CH_2) str., 1606 (C=N) str., 1514 Ar(C=C) str., 1255 (asym. C-O-C) str. for ethoxy gr., 1037 (sym. C-O-C) str. for ethoxy gr., 631 (C-S) str., 1531 (asym. NO_2) str.,1338 (sym. NO_2) str; **^{1}HNMR (400MHz),DMSO-d_6,δ ppm):** 8.95-7.61 (m, 10H, Ar-H), 6.66 (s,1H, CH=C-), 4.00(q,2H,CH_2), 3.76 (s, 3H, OCH_3), 1.32(t,3H, CH_3); Elemental analysis Calcd. for $C_{25}H_{20}N_6O_6S$: C, 56.39; H, 3.79; N, 15.78; S, 6.02. Found: C, 56.613; H, 3.017; N, 15.550; S, 6.159.

Synthesis of N-(2,4-dinitrophenyl)-4-[(5-ethoxy-1H-benzo[d]imidazole-2-yl)thio]-3-(4-nitrophenyl)-1H-pyrazole-1-amine (6d):

Green powder, Yield 83%; m. p. 92-94 °C; **IR (υ= cm^{-1}, KBr):** 3089 Ar(CH) str., 2978 (asym. CH_3) str., 1606 (C=N) str., 1516 Ar(C=C) str.,

1257 (asym. C-O-C) str. for ethoxy gr., 1037 (sym. C-O-C) str. for ethoxy gr., 640 (C-S) str., 1531 (asym. NO_2) str., 1336 (sym. NO_2) str; **^{1}HNMR (400MHz), DMSO-d_6, δ ppm):** 8.98-7.03 (m,10H, Ar-H), 6.66 (s,1H, CH=C-), 4.01(q,2H,CH_2), 1.33(t,3H, CH_3); Elemental analysis Calcd. for $C_{24}H_{17}N_7O_7S$: C, 52.65; H, 3.13; N, 17.91; S, 5.86. Found: C, 52.198; H, 3.99; N, 18.063; S, 5.953.

2.7. General method for the synthesis of 2-[(1-benzoyl-5-ethoxy-1H-benzo[d]imidazole-2-yl)thio]-1-(4-substituted phenyl)ethanone (7a-d) [23].

An equimolar mixture of the compounds **(4a-d)** (0.01mol),(4a:3.12g, 4b:3.46g, 4c:3.42g, and 4d: 3.57g), and benzoyl chloride (1.16ml, 0.01mol) in an aqueous NaOH (10%) solution stirred for 10-12 h at room temperature. A solid ppt., that separated filtered off and washed with dil. HCl recrystallized from ethanol.

Synthesis of 2-[(1-benzoyl-5-ethoxy-1H-benzo[d]imidazole-2-yl)thio]-1-phenylethanone(7a):

Gray powder, Yield 88%; m. p. 135-137 °C; **IR (υ= cm^{-1}, KBr):** 3072 Ar(CH) str., 2978 (asym. CH_3) str., 2877 (sym. CH_3) str., 2839 (sym. CH_2) str., 1687 (C=O) str., 1637 (C=N) str., 1600, 1581 Ar(C=C) str., 1217 (asym. C-O-C) str., 1045 (sym. C-O-C) str., 665, 623 (C-S) str; **^{1}HNMR (400MHz),DMSO-d_6, δ ppm):** 7.97-6.65 (m, 13H,Ar-H), 6.60 (s,2H, CH_2-S),4.03(q,2H,CH_2), 1.32 (t,3H,CH_3); Elemental analysis Calcd. for $C_{24}H_{20}N_2O_3S$: C, 69.21; H, 4.84; N, 6.73; S, 7.70. Found: C, 69.862; H, 4.738; N, 6.368; S, 7.906.

Synthesis of 2-[(1-benzoyl-5-ethoxy-1H-benzo[d]imidazole-2-yl)thio]-1-(4-chlorophenyl)ethanone (7b):

Greenish yellow powder, Yield 82%; m. p. 142-144 °C; **IR (υ= cm^{-1}, KBr):** 3076, 3059 Ar(CH) str., 2974 (asym. CH_3) str., 2877 (sym. CH_3) str., 2841 (sym. CH_2) str., 1680 (C=O) str., 1635 (C=N) str., 1591 Ar(C=C) str., 1259 (asym. C-O-C) str., 1041 (sym. C-O-C) str., 667, 626 (C-S) str., 584 (C-Cl) str; **^{1}HNMR(400MHz),DMSO-d_6 δ ppm):** 7.96-7.56 (m,12H,Ar-H),6.50(s,2H,CH_2-S). 4.03 (q, 2H, CH_2), 1.30(t, 3H, CH_3); Elemental analysis Calcd. for $C_{24}H_{19}ClN_2O_3S$: C, 63.92; H, 4.25; N, 6.21; S, 7.11. Found: C, 63.147; H, 4.362; N, 6.512; S, 7.018.

Synthesis of 2-[(1-benzoyl-5-ethoxy-1H-benzo[d]imidazole-2-yl)thio]-1-(4-methoxyphenyl)ethanone (7c):

Beige powder, Yield 56%; m. p. 148-150 °C; **IR (υ= cm^{-1}, KBr):** 3080, 3059 Ar(CH) str., 2972 (asym. CH_3) str., 2877 (sym. CH_3) str., 2841 (sym. CH_2) str., 1685 (C=O) str., 1637 (C=N) str., 1602, 1577 Ar(C=C) str., 1265 (asym. C-O-C) str. for ethoxy gr., 1043 (sym. C-O-C) str. for ethoxy gr., 1222 (asym. C-O-C) str. for methoxy gr., 630, 617 (C-S) str; **^{1}HNMR (400MHz), DMSO-d_6, δ ppm):** 7.91-6.65 (m, 12H, Ar-H), 4.02(q,2H,CH_2), 3.83 (s,3H,OCH_3),1.32(t,3H,CH_3); Elemental analysis Calcd. for $C_{25}H_{22}N_2O_4S$: C, 67.25; H, 4.97; N, 6.27; S, 7.18. Found: C, 67.019; H, 5.453; N, 6.644; S, 7.429.

Synthesis of 2-[(1-benzoyl-5-ethoxy-1H-benzo[d]imidazole-2-yl)thio]-1-(4-nitrophenyl)ethanone (7d):

Beige powder, Yield 59%; m. p. 175-177 °C; **IR (υ= cm^{-1}, KBr):** 3111, 3078 Ar(C-H) str., 2877 (sym. CH_3) str., 2837 (sym. CH_2) str., 1691 (C=O) str., 1635 (C=N) str., 1602 Ar(C=C) str., 1222 (asym. C-O-C) str., 1043 (sym. C-O-C) str., 661,623 (C-S) str., 1523 (asym. NO_2) str., 1344 (sym. NO_2) str; **^{1}HNMR (400MHz), DMSO-d_6,δ ppm):** 8.34-6.65 (m, 12H, Ar-H), 4.02(q,2H,CH_2), 1.32(t,3H,CH_3); Elemental analysis Calcd. for $C_{24}H_{19}N_3O_5S$: C, 62.46; H, 4.15; N, 9.11; S, 6.95. Found:C, 62.313; H, 4.960; N, 9.004; S, 6.622.

2.8. Synthesis of 2-[(1-acetyl-5-ethoxy-1H-benzo[d]imidazole-2-yl)thio]-1-phenylethanone)(8) [24].

A mixture of (**4d**),(0.01mol, 3.57g) and Ac_2O (10 ml) was stirred at room temperature for 3h. The resulting precipitate collected by filtration and recrystallized from ethanol to afford green powder **(8)**. Yield 78%; m. p. 238-240 °C; **IR (υ= cm^{-1}, KBr):** 3010 Ar(CH) str., 2941(asym. CH_3) str., 2929 (asym. CH_2) str., 2883 (sym. CH_3) str., 2835 (sym. CH_2) str., 1695 (C=O) str., 1635 (C=N) str., 1600, 1558 Ar(C=C) str., 1261 (asym. C-O-C) str., 1043 (sym. C-O-C) str., 659, 623 (C-S) str., 1525 (asym. NO_2) str., 1346 (sym. NO_2) str; **^{1}HNMR (400MHz), DMSO-d_6,δ ppm):** (8.40, d, 2H,Ar-H),8.30 (d,2H Ar-H), 7.29 (d,1H,Ar-H_7), 6.91(d,1H,Ar-H_4), 6.73 (d, 1H, Ar-H_6), 5.05 (s,2H, CH_2-S), 4.01(q,2H, CH_2), 2.10 (s.3H, CH_3-C=O), 1.32(t,3H, CH_3); Elemental analysis Calcd.for $C_{19}H_{16}N_3O_5S$: C, 57.28; H, 4.04; N, 10.55; S, 8.05. Found: C, 57.154; H, 3.808; N, 10.463; S, 7.851.

2.9. Antimicrobial Activity and Sensitivity Assay [25, 26]

The synthesized compounds have been studied for their antibacterial activity *in vitro,* against four tested bacteria (*Staphylococcus aureus, Streptococcus agalactiae* as Gram-positive bacteria and *Pseudomonas aeruginosa and Proteus mirabilis*, as Gram-negative bacteria) and maintained on nutrient agar medium for further study. Imipenem and cefotaxime were used as standard drugs for antibacterial activity.

The antimicrobial activity was evaluated by Agar Diffusion Well method .The indicator microorganisms were plated on Mueller-Hinton agar plates containing 4 orifices of 6 mm, where 100 μL of the suspension of compounds (50, 100 mg/ml of 25% dimethyl sulfoxide) was placed in each well and standardized according to the McFarland scale (tube 0.5). The diameter of the zones of inhibition (ZI) measured in (mm) after 24 hours of incubation at 37°C, table 2.

Table (1) : The melting points, R_f values, % of yield and Physical appearance of the title compounds

Comp. No	Chem. formula	m.p	% Yield	*R_f	Physical appearance	Solvent of recrystallization
1a	$C_{10}H_{12}N_2OS$	94-96	76	0.46[g]	Light brown powder	Ethanol
1b	$C_{12}H_{16}N_2OS$	124-126	61	0.52[g]	Beige powder	Ethanol
1c	$C_{16}H_{16}N_2OS$	89-91	91	0.86[b]	Brown crystals	Ethanol
2	$C_{20}H_{22}N_2O_4S$	161-163	74	0.67[d]	Light beige powder	Ethanol
3	$C_{15}H_{18}N_2OS$	240-242	86	0.57[c]	Off Black powder	Ethanol
4a	$C_{17}H_{16}N_2O_2S$	203-205	94	0.60[e]	Gray powder	Acetone
4b	$C_{17}H_{15}ClN_2O_2S$	231-233	92	0.57[e]	Gray powder	Acetone
4c	$C_{18}H_{18}N_2O_3S$	205-207	89	0.52[e]	Blue powder	Acetone
4d	$C_{17}H_{15}N_3O_4S$	246-248	96	0.47[e]	Beige powder	Acetone
5a	$C_{23}H_{20}N_6O_5S$	221-223	85	0.62[h]	Bright green powder	Ethanol
5b	$C_{23}H_{19}ClN_6O_5S$	198-200	79	0.54[h]	Bright green powder	Ethanol
5c	$C_{24}H_{22}N_6O_6S$	188-190	83	0.42[h]	Yellowish green powder	Ethanol
5d	$C_{23}H_{19}N_7O_7S$	240-242	90	0.38[h]	Bright gray powder	Ethanol
6a	$C_{24}H_{18}N_6O_5S$	105-107	60	0.65[a]	Yellow powder	Ethyl acetate
6b	$C_{24}H_{17}ClN_6O_5S$	108-110	58	0.48[a]	Yellowish green powder	Ethyl acetate
6c	$C_{25}H_{20}N_6O_6S$	116-118	49	0.53[a]	Bright green powder	Ethyl acetate
6d	$C_{24}H_{17}N_7O_7S$	92-94	83	0.66[a]	Green powder	Ethyl acetate
7a	$C_{24}H_{20}N_2O_3S$	135-137	88	0.31[d]	Gray powder	Ethanol
7b	$C_{24}H_{19}ClN_2O_3S$	142-144	82	0.33[d]	Greenish yellow powder	Ethanol
7c	$C_{25}H_{22}N_2O_4S$	148-150	56	0.34[d]	Beige powder	Ethanol
7d	$C_{24}H_{19}N_3O_5S$	175-177	59	0.68[d]	Beige powder	Ethanol
8	$C_{19}H_{16}N_3O_5S$	238-240	78	0.43[f]	Green powder	Ethanol

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

a.: n-hexane: ethyl acetate (6:4); b: chloroform: methanol (8:2); c: n-hexane: ethyl acetate(4:6); d: chloroform: methanol (9:1); e: chloroform: acetone (9:1); f: chloroform: acetone (8: 2); g: chloroform: acetone (9.5: 0.5); h: n-hexane: acetone (9.5: 0.5)

3. RESULTS AND DISCUSSION

3.1. Chemistry

The synthesis of the title compounds **1-8** was accomplished and outlined in the scheme **1.**

The scheme**1** illustrated the reactions sequences for all synthesized derivatives.The first step demonstrates the reaction of the 5-ethoxy-2-MBI with different alkyl halides to produce **1a-c**. While in the second step is the reaction of 5-ethoxy-2-MBI with ethyl bromoacetate in the presence of a base to form ethyl ester derivative, then the treatment with the benzoyl chloride to afford the compound **2.**

The compound **3** obtained in a good yield by refluxing the 5-ethoxy-2MBI with an aliphatic ketone (4-methyl-2-pentanone) using acidified acetic acid solution.

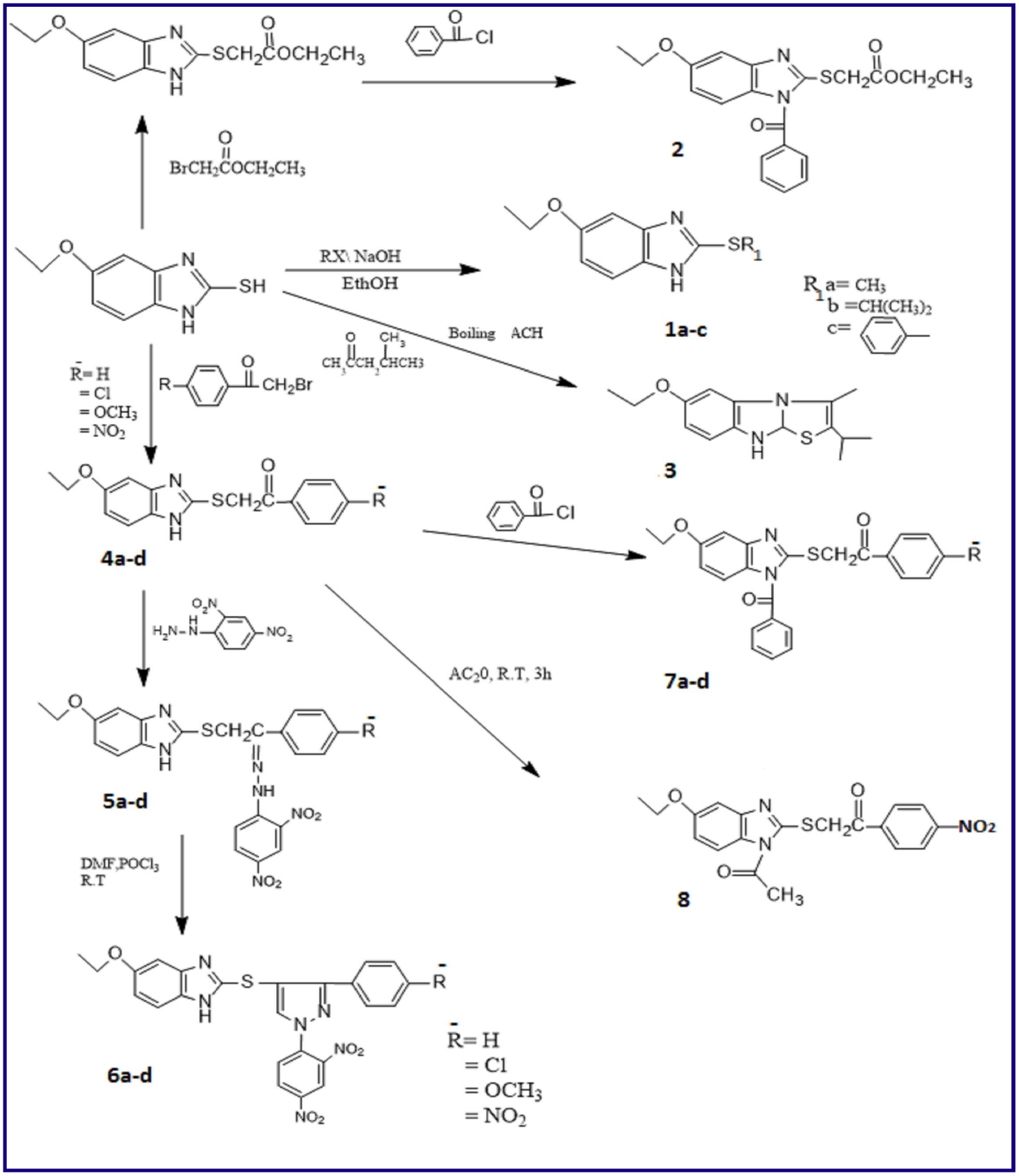
On the other hand, the reaction of 5-ethoxy-2-MBI with different *p*-substituted phenacyl bromide derivatives, by refluxing the mixture in a basic medium, furnish the 5-ethoxy-2-MBI acetophenone derivatives **4a-d.**

Similarly, the reaction of 5-ethoxy-2-MBI **4a-d** with 2,4-di-nitrophenyl hydrazine in acidic medium, produced Schiff bases, **5a-d**, which then treated with phosphorous oxychloride in DMF, (vilsmier Haack) reaction afforded the cyclized pyrazole derivatives **6a-d.**

Finally, the compounds **4a-d** when stirred at R.T with benzoyl chloride, and acetic anhydride, produced the compounds **7a-d** and **8**, respectively**.**

The IR absorption bands that present in all title compounds including the asymmetric and symmetric $\underline{CH_3}$ stretching vibrations at (2980-2950) $cm^{-1}$and (2880-2860) cm^{-1}, respectively. While the aromatic (CH) stretching showed, distinctive bands at (3100-3010) cm^{-1}, other bands at (1647-1616) cm^{-1} for aromatic (C=N) stretching, different absorption bands ranged at (1602-1510) cm^{-1} accounted for aromatic (C=C) stretching.

Also, important bands displayed at (1274, 1216) cm^{-1} for the asymmetric and symmetric (C-O-C) stretching of the ethoxy group, respectively, and the appearance of two medium stretching (C-S) absorption bands at (682 and 623) cm^{-1}. for the newly synthesized derivatives.



Scheme (1): - Synthesis of 5-ethoxy-2MBI derivatives.

The compound **1a**, showed the presence of absorption bands of the asymmetrical and symmetrical (CH_3) stretching at (2970 and 2881) cm^{-1}, respectively, and the appearance of the band at (673) cm^{-1} due to (C-S) stretching.

The compound **1b**, illustrated the appearance of a medium absorption bending band at (1396) cm^{-1}, due to the presence of the geminal methyl groups ($CH(CH_3)_2$).

The compound **1c** showed aromatic protons at 3059 cm^{-1} and asymmetrical and symmetrical stretching bands for the methyl group (CH_3) at (2980,2870) cm^{-1}, respectively.

While the compound **(2),** demonstrated important peaks at (2978, 2879) and (2929, 2820) cm^{-1} due to asymmetrical and symmetrical (CH_3) and (CH_2) stretching bands, respectively, also the presence of a characteristic band at (1710) cm^{-1}, accounted for the aliphatic (C=O) stretching for the ester group.

The IR spectrum for compound **(3)** showed the aromatic (CH) stretching bands at (3088, 3064) cm^{-1}, in addition, the appearance of two absorption bending bands at (1379 and 1363) cm^{-1} due to the geminal methyl group, ($CH(CH_3)_2$, that is attached to the thiazole ring present in the parent nucleus.

The IR spectra for the compounds **4a-d**, demonstrated importantly (C=O) stretching bands at (1672, 1674, 1660, and 1695) cm^{-1} respectively, in addition, different peaks displayed at (1618-1518) cm^{-1}, due to the (C=C) stretching.

It is important to mention the disappearance of the (C=O) stretching bands in the compounds **(5a-d)** and the appearance of the absorption bands at (1614 and 1616) cm^{-1} due to the formation of the aliphatic (C=N) stretching.

The IR spectra for the compounds **5a-d** showed distinctive peaks at 1616 cm^{-1} accounted for the Schiff bases,(C=N). The IR spectra for the compounds **6a-d**, illustrated important bands at (1614 - 1606) cm^{-1} due to the aromatic (C=N) stretching.

In addition, The IR spectra for the compounds **7a-d** showed absorption bands at (1687, 1680, 1685, and 1691) cm^{-1} respectively, attributed to (C=O) stretching for ketone. While, the IR spectra for the compound **(8)**, illustrated the appearance of the distinctive band at (1695) cm^{-1}, due to conjugated (C=O) stretching.

The IR spectra for the compounds, **4b, 5b, 6b** and **7b** demonstrated two small bands ranged at (640-584) cm^{-1}, due to (C-Cl) stretching. Finally, there are two stretching absorption bands for (NO_2) group, at (1531-1518) cm^{-1}) due to (asymmetrical NO_2) stretching and at (1346cm^{-1}) accounted for the (symmetrical NO_2) stretching band present in the IR spectra for the compounds **4d, 5d, 6d, 7d,** and **8**.

The ^{1}HNMR spectra for the synthesized derivatives showed the following distinctive peaks, The compound **1a** displayed prominent singlet peak at δ = 2.66 ppm attributed to CH_2-S, while **1b** showed a peak as a multiplet at δ=3.88 ppm due to **CH**$(CH_3)_2$, and a peak attributed to the geminal methyl groups $CH(\mathbf{CH_3})_2$, as a doublet, at δ = 1.35 ppm. Moreover, **1c** displayed characteristic peaks attributed to the aromatic benzoyl protons, as a multiplet, at δ =7.33-7.23 ppm. Compound **2** displayed characteristic peaks, as a multiplet, attributed to the aromatic protons at δ = 7.49- 7.46 ppm.

The ^{1}HNMR for **3** showed a peak, as a multiplet, at 3.41 ppm, attributed to $CH(CH_3)_2$, other singlet peak attributed to (CH_3) attached to the thiazole ring at δ = 2.47 ppm, and a prominent peak, as a doublet, accounted for the geminal methyl groups, $CH(CH_3)_2$.

Compounds **4a-d**, that result from the reaction of 5-ethoxy-2MBI with substituted phenacyl bromides in dry acetone containing K_2CO_3 as a base, refluxed for 3h; showed a distinct peak at δ= 5.70-5.18, as a singlet, accounted for CH_2-S.

The ^{1}HNMR for **5a-d**, illustrated a distinctive peak, as a singlet, due to the NH- at δ = 11.16-10.82 ppm. While, the compounds **6a-d**, exhibited characteristic peak appeared as a singlet, due to CH=C-, at δ= 6.70- 6.66 ppm.

Moreover, The compounds **7a-d** demonstrated distinctive peaks accounted for the aromatic protons, at 8.43-6.65 ppm.

Finally, the compound **8** result from acylation of **4d** with AC_2O at R.T for 3h,showed a characteristic peak at δ=2.1 ppm, as a singlet due to CH_3-C=O.

All the title compounds **1-8**, exhibited distinctive peaks, attributed to the ethoxy group as a triplet, at δ= 1.36-1.30 ppm, and for the (CH_2) group as a quartet displayed at δ= 4.08- 3.98 ppm. The compounds **4c, 5c, 6c** and **7c** illustrated a peak, as a singlet, at δ= 3.88- 3.76 ppm. due to the methoxy group present at the *para* position of the aromatic ring.

3.2. *Antimicrobial assay*

The antimicrobial evaluation of the synthesized compounds was done using well diffusion technique for Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus agalactiae)* and Gram-negative bacteria (*Proteus mirabilis* and *Pseudomonas aeruginosa*) in comparison with cefotaxime and imipenem as positive control and DMSO as negative control, as shown in table **2**.

Table (2): The antibacterial activity of tested compounds.

Comp.No.	Conc. In µg/ml	*Staphylococcus aureus*	*Streptococcus agalactiae*	*Proteus mirabilis*	*Pseudomonas aeruginosa*
		Inhibition zone in (mm)			
1a	50	13	10	7	5
	100	21	20	17	6
1b	50	1	0	0	0
	100	6	3	0	0
1c	50	11	5	2	0
	100	23	15	5	2
R2	50	11	9	6	4
	100	17	15	11	10
3	50	15	9	4	3
	100	29	17	10	7
5a	50	4	2	0	0
	100	8	7	3	0
5b	50	3	2	0	0
	100	5	4	4	2
5c	50	1	0	0	0
	100	5	3	2	0
5d	50	7	6	2	0
	100	15	11	4	0
6a	50	14	8	7	4
	100	21	18	16	7
6b	50	21	12	6	2
	100	36	32	11	8
6c	50	10	7	6	2
	100	16	13	11	9
6d	50	14	8	7	4
	100	22	21	13	9
7a	50	14	6	2	0
	100	21	19	11	4
7b	50	20	13	9	5
	100	37	30	23	21
7c	50	14	10	7	6
	100	25	22	19	11
7d	50	13	10	6	2
	100	22	22	18	7
8	50	7	2	0	0
	100	14	6	3	0
*cefotaxime	10	44	40	32	26
imipenem	10	48	42	35	29
**DMSO	25%	0	0	0	0

**cefotaxime and imipenem as positive control, **DMSO as negative control diluted to 25%*

From the above results, we can conclude the following:

All the synthesized compounds exhibit various antibacterial activities against the Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus agalactiae*) and Gram-negative bacteria (*Proteus mirabilis, Pseudomonas aeruginosa*) at a concentration of 100 µg/ml.

The compound **1a** and **1c** showed the highest antibacterial activity (ZI: 21, 20mm), and (23 mm and 15 mm) at a concentration of 100 µg/ml. against *Staph. aureus* and *Streptococcus agalactiae*, respectively.

The compound **2** showed potent antibacterial activities against Gram-positive *Staph.aureus* and *Strepto. agalactiae* at a concentration of 100 µg/ml.

The compound **3** exhibited a potent antibacterial activity against *Staph. aureus* and a highest activity against Gram–positive *Strepto. agalactiae*. at a concentration of 100 µg/ml.

The compounds **6a** showed distinctive antibacterial activity against *Staph.aureus*, (ZI:21mm), *Strepto. agalactiae* (ZI:18mm), and remarkable antibacterial activity against *Proteus mirabilis*, (Z1:16mm) at a concentration of 100 µg/ml.

-The compounds **6b** and **7b** showed the greatest antibacterial activities against Gram-positive (*Staphylococcus aureus* (ZI:36mm, 37mm), respectively, and *Streptococcus agalactiae* (ZI:32mm, 30mm), respectively, at a concentration of 100µg/ml.

The compounds **7a-7d** demonstrated various potent activity against *Staph. aureus* and *Strepto. agalacitae* at a concentration of 100µg/

ml, especially Compound **7b** showed distinctive antibacterial activities against both Gram-positive and Gram-negative bacteria, (see table **2**). The rest of tested compounds showed slight to moderate activities against Gram –positive *Staph. aureus* and *Strepto. agalactiae.*

4. CONCLUSION

In this study, we report the synthesis of new derivatives of 5-ethoxy-2-mercapto benzimidazole and evaluated for their antibacterial activities. Some of the tested compounds have potent antibacterial activities, especially against Gram-positive bacteria. The compound **6b** exhibited the highest antibacterial activity against *Staph. aureus*, while the compound **7b** demonstrated the highest antibacterial activity against Gram-negative bacteria, *Proteus mirabilis,* and *Pseudomonas aeruoginosa*.

Acknowledgement

The authors would like to thank the college of pharmacy/Dept.of pharmaceutical chemistry-Baghdad for support and carrying out the research; also a great appreciation and gratitude to Dr. Linda H. Turkey for performing the antibacterial work.

Conflict of interest: The authors declare no conflict of interest.

REFERENCES

1. M. J. Aaglawe, S. S. Dhule, S. S. Bahekar, P. S. Wakte, and D. B. Shinde; Synthesis and antibacterial activity of some oxazolone derivatives, *J. of the Kor. Chem. Soc*., 2003, 47(2), 1-4
2. Bagher Eftekhari and Maryam Zirak; Chemistry of α Oxoesters: A powerful tool for the synthesis of heterocycles, *pubs. acs. org*, 2014, page 2
3. Ramanpreet Walia, Md. Hedaitullah, Syeda Farha Naaz, Khalid Iqbal and HS. Lamba; benzimidazole derivatives: an overview, *I. J. R. P. C*., 2011, 1(3), 565-574
4. Bhanupriya Bhrigu, Nadeem Siddiqui, Devender Pathak, M. Shamsher Alam, Ruhi Ali, and Bishmillah Azad; Anticonvulsant evaluation of some newer benzimidazole derivatives: design and synthesis, *Act. Pol. Phar. D. Res*., 2012, 69 (1), 53-62
5. Sreenivasulu Enumula, Anees Pangal, Muiz Gazge, Javed A. Shaikh and Khursheed Ahmed; Diverse pharmacological aspects of benzimidazole derivatives: A Review, *Res. J. Chem. Sci.*, 2014, 4(4), 78-88.
6. Roberto P. Ginni, Ettore Parini, Giancarlo Tonon; Pharmacologically active alkylthi- benzimidazole derivatives and process for the preparation thereof, Uni. St. Patent, 1991, 4, 983, 620.
7. Hassan Y Aboul-Enein1 and Ahemd A El Rashedy; Benzimidazole derivatives as antidiabetic agents, *Med. Chem. J.*, 2015, 5(7), 318-325
8. E. Abele, R. Abele, P. Arsenyan, S. Belyakov, M. Veveris, and E. Lukevics; Phase transfer catalytic synthesis and hypocholesterolemic activity of thiazino[3,2-a]benzimidazole and its silicon analoge, *Chem. of Het. Com*., 2007, 43(2), 220-224
9. S. E. Milkina, O. B. Stepanenko, L. N. Grushevskaya, N. I. Avdyunina, B. M. Pyatin, V. L. Bagirova and E. B. Nechaeva; Analysis and standardization of the new anxiolytic drug afobazole, *Pha. Chem. J*., 2006, 40(7), 405-406
10. Hatem A. Abdel-Aziz, Tamer S. Saleh, and Heba S. A. El-Zahabi; Facile synthesis and in-vitro antitumor activity of some pyrazolo[3,4-b]pyridines and pyrazolo[1,5-a]pyrimidines linked to a thiazolo[3,2-a]benzimidazole moiety, *Arch. Pharm. Chem. Life Sci.* 2010, 343, 24–30
11. Ramya V. Shingalapur, Kallappa M. Hosamani, Rangappa S. Keri, Mallinath H. Hugar; Derivatives of benzimidazole pharmacophore: Synthesis, anticonvulsant, antidiabetic and DNA cleavage studies, *Eur. J. of Med. Chem.* , 2010, 45, 1753–1759
12. Sidram A. Nevade, Sachin G. Lokapure, and Navanath V. Kalyane; Synthesis and pharmacological evaluation of some novel 2-mercapto benzimidazole derivatives, *J. of Kor. Chem*., 2013, 57(6), 755-760
13. Gangula Mohan Rao1, Yellu Narasimha Reddy and Baru Vijaya Kumar; Evaluation of analgesic and anti-inflammatory activities of N-mannich bases of substituted 2-mercapto-1H-benzimidazoles, *Int. J. of Ap. Bio. and Pha. Tec*., 2013, 4(1), 38-46
14. Sergiy Oliynyk and Seikwan Oh; The pharmacology of actoprotectors: Practical application for improvement of mental and physical performance, *Bio. Mol. Ther.*, 2012, 20(5), 446-456.
15. Jabali J. Vora, Keyur P. Trivedi and Rahul S. Kshatriya; An improved synthesis of 2-mercapto-5-difluoromethoxy-1H benzimidazole: An important medicinal intermediate, *Adv. Appl. Sci. Res*., 2011, 2(3), 89-93.
16. Anisetti Ravinder Nath and Malladi Srinivas; Design, synthesis, antibacterial and antifungal activity of novel 2-[(E)- 2-aryl-1-ethenyl]-3-(2-sulfanyl-1H-benzo [d]imidazole-5-yl)-3,4- dihydro-4-quinolinones, *E. J. of Chem*., 2012, 9(3), 1481-1489.

17. Gigani Yaseen and Jaduav Sudhakar; Design, synthesis and antimicrobial activity of 2-mercapto benzimidazole derivatives, *Int. J. of Pha. and Bio Sc*., 2010, 1(4), 281-286

18. Mohammed R. Ahamed, Shetha F. Narren and Amal S. Sadiq; Synthesis of 2-mercapto benzimidazole and some of its derivatives, *J. of Al-Nahrain Uni*., 2013, 16(2), 77-83

19. Rafah F. Al-Smaisim, Ayad M., R. Raauf, Afrah Mohammed, Hassan Salman; Synthesis, characterization and antimicrobial activity of some new 2-mercapto benzothiazole heterocyclic derivatives, *Med. J. of Babylon*, 2010, 7(4,3), 422-430

20. Khalid A. Al-Rashood and Hatem A. Abdel-Aziz; Thiazolo[3,2-a]benzimidazoles: Synthetic strategies, chemical transformations and biological activities, *Mol*., 2010, 15, 3775-3815.

21. Yasmeen A. Al-Daeif; Synthesis of bis-heterocyclic phenyl containing -4 – oxo – 1,3 – thiazolidine, 2,3 – dihydrothiazole, 1, 2,4 – triazolo [3, 4b]–1,3,4 – thiadiazine and pyrazole derivatives, *J. of Al-Nah. Uni*. , 2011,14 (2), 67-74.

22. S. Srinivas Rao, Putluru Mahesh, Ch Venkata Ramana Reddy & P K Dubey; Synthesis of N-alkylated-2-(1,3-diphenyl-1H-pyrazole-4-ylsulfanyl)-1H-benzimidazoles by Vilsmeir-Haack reaction and by condensation with DMF-DMA, *Ind. J. Chem*., 2015, 54B, 531-537

23. Sandeep Gupta, S. S. Pancholi, and M. K. Gupta; Synthesis and antimicrobial screening of some acid chloride derivatives of 2-substituted benzimidazoles, Ori. *J. of Chem*. , 2009, 25(1), 219-222.

24. Abd EI-Wareth A.O. Sarhan, Hasan A.H. El-Sherief, and Abdalla M. Mahmoud; A convenient one-pot synthesis of 2-benzimidazolyl thioacetophenones and thiazolo[3,2-a] benzimidazoles, *Tet*., 1996, 52(31), 10485-10496.

25. S. Febina Bernice Sharon, Rashel Regil Daniel and R. Shenbagarathia: Optimization of antibiotic production by marine actinomycetes Streptomyces spp.Kod10, *Int. J. Pharm. Sci*., 2014, 6(2), 506-510

26. Ingrid Reis da Silva, Mayra Kassawara Martins, Clarice Maia Carvalho, João Lúcio de Azevedo, and Rudi Emerson de Lima Procópio; The effect of varying culture conditions on the production of antibiotics by Streptomyces spp. isolated from the Amazonian soil, *Fer. Tec. J.,* 2012, 1(3), 1-5.

Source of support: Nil, India**, Conflict of interest:** None Declared