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Facile Synthesis and In-Vitro Antimicrobial Activity of Some Novel 2-Hetroamido-5-Amino Benzimidazoles

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ABSTRACT

A series of novel benzimidazoles derivatives were synthesized in convenient, easy and cheap way. These benzimidazoles are characterized by bearing amino substituents (morpholine and N-methylpiperazine) at positions 5, in addition to pyrimidine, pyridine, furyl, thienyl and pyryl with amide linkage at positions 2. The methodology of such synthetic routes was represented in synthesis of novel structures that the microbes have never been presented with before; that would hopefully prevent the process in which microbes resist antimicrobial drugs. The structures of all new compounds were identified by ^{1}H-NMR, ^{13}C-NMR, M.S and FT-IR spectroscopic techniques and elemental analysis. All the compounds synthesized in this work were examined for their in vitro antimicrobial activities against Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*), and the fungi (*C.albicans and A.niger*). Compared to Ciprofloxacin and Fluconazole as the reference substances, some of the synthesized compounds showed high antibacterial and antifungal activities against studied strains with inhibition zones between (12-27) mm.

Keywords: benzimidazole, antibacterial activity, antifungal activity, 2,5-disubstituted benzimdazole

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INTRODUCTION

A huge number of antimicrobial agents are used nowadays to treat microbial infections, but still little number of such drugs can be classified as efficient, proper and fit one. Most of the current drugs are toxic, enable recurrence because of their bacteriostatic and fungistatic feature and not bactericidal/fungicidal or might lead to resistance due to prolonged periods of administration[1,2]. Benzimidazole derivatives are very useful compounds for the development of molecules of pharmaceutical or biological interest. Substituted benzimidazole derivatives have found applications in diverse therapeutical areas including antimicrobial[3-5], antioxidant[6], antiviral[7,8], antihypertensive[9], antiprotozoal[10] , antiulcer[11],anti-inflammatory [12] and molluscicidal agents[13]. Furthermore, benzimidazoles showed anticancer activity against DNA topoisomerase I[14,15]and colon cancer cell lines[16]. Since having pharmacophore and privileged structure in medicinal chemistry including a diverse level of antimicrobial activity; a tangible need to optimize and discover new structural benzimidazole family-as one of the most versatile class of compounds against microbes- is still under focusing for many researchers [4,9]. For that many publications have revealed that the substitutions at the 1, 2 and 5 positions of the benzimidazole moiety are crucial for exhibiting a wide range of pharmacological activities such as the synthesis of derivitized benzimidazole through introducing amide and amino linkage with various heterocyclic nucleui (pyridine, N-methyl-pyrrole or imidazole) at C-2 and C-5 and evaluated their anti-tumor and anti-viral activities. The main twoapproaches to develop new antimicrobial drugs are; either synthesis of analogues or searching novel structures that the microbes have never been presented with before.[19]

For that Ten of novel benzimidazoles derivatives bearing morpholine and N-methylpiperazine at positions 5, in addition to pyrimidine, pyridine, furyl, thienyl and pyrrole with amide linkage at positions 2 were synthesized based on simple functionality reactions that were used towards having not only cheap and easy synthetic approach but also beneficial targets. Figures 1-2 showall synthesized compounds (10a-14a) and (10b-14b) with full description of structures, reagents and reactions conditions.

MATERIALS AND METHODS:

Chemistry

^{1}H NMR spectra were recorded at 400 MHz on a Bruker DPX 400 spectrometer and ^{13}C NMRspectra at 75 MHz spectrometer. Chemical shifts are denoted in ppm (δ) relative to internal solvent standard (Me_4Si in the case of ^{1}H). The splitting patterns for NMR-spectra are designated as follows: s (singlet), d (doublet),dd (doublet of doublet), t (triplet),br (broad) and m (multiplet). Coupling constants (J) are designated in Hz.Mass spectra were recorded on a VG 70/70 Hybrid or a Kratos MS-50 mass spectrometer by ES. Elemental analyses were performed using a LECO CHNS-932 elemental analyzer. IR spectra were obtained using a Perkin Elmer 1605 FTIR spectrometer. Analytical TLC was performed on Merck 60 F254 aluminium backed plate and were developed using an UV lamp. All chemicals were used directly from supplier's vessel without further purification.

Step1:- General procedure for the Synthesis of 2a and 2b

A mixture of compound (1); 4-fluoro-1,2-dinitrobenzene (5 mmol), $NaHCO_3$ (10 mmol) and methanol (30 mL) was refluxed for 30 minutes, the appropriate amine (N-Methylpiperazine or Morpholine) (5 mmol) was added, the colorless solution became yellow. The reaction mixture was heated under reflux until TLC showed complete disappearance of the starting material. The reaction mixture was cooled in an ice bath for 2 hours, and the yellow product was separated from methanol by filtration. This precipitate was washed with (2 X 20 mL) of ethyl acetate, HCl (10%), brine (10 mL) and water(10 mL), and the organic layer dried ($MgSO_4$) and recrystallized from ethanol affording the dinitro compounds 2a and 2b asillustrated in **Figure 1**.

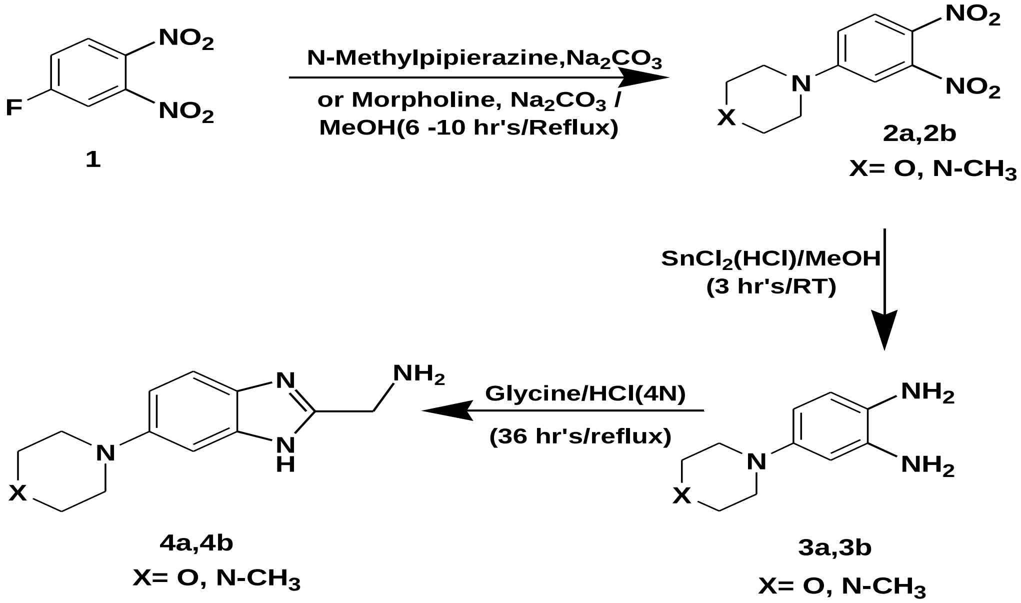


Figure (1):- Synthetic route of 2-(aminomethyl)-5-morpholiny and 2-(aminomethyl)-5-piperazinyl benzimidazoles (4a and 4b)

Step 2:- General procedure for the Synthesis of 3a and 3b

A solution of the dinitro 2a or 2b (2 mmol) in conc. HCl (10 mL) was treated portion wise with $SnCl_2$ (24 mmol.) and the resulting solution was stirred at room temperature for several hours. The reaction mixture was poured into ice-water and neutralized with NaOH 10% solution, extracted with ethyl acetate (5 X 25 mL) washed with water (3 X 15 mL) dried (Na_2SO_4), and recrystallized from ethanol affording the dinitro compounds 3a and 3b as illustrated in Figure 1.

Step 3:- General procedure for the Synthesis of 4a and 4b

A solution of *o*-diamine (10 mmol) and 4N HCl (15 mL) was treated with glycine (15 mmol) and heated under reflux[18]. The solution was cooled and allowed to stand overnight. The mixture was neutralized with 2 N NaOH, the solid was filtered, washed with water and purified by recrystallization from ethanol affording compounds 4a and 4b asillustrated in Figure 1.

Step 4:- General procedure for the Synthesis of compounds (10a-14a) and (10b-14b)

Appropriate carboxylic acid 5,6,7,8 and 9 (1.1 mmol) was dissolved in dry dimethylformamide (DMF), (10 mL) with HOBt (1.1 mmol) and EDC(1.1 mmol). The reaction was then cooled to 0°C for 30 minutes then aminobenzimidazoles 4a and 4b (1 mmol) was added and the reaction left stirring at room temperatureas illustrated in Figure 2. Once the TLC showed no starting material (4-6 hours); the solvent was removed in *vacuo*, then (25 mL) water and (50 mL) ethyl acetate were added and the organic layer was washed with HCl 5% (5mL) then with $NaHCO_3$ (10%) (5mL) and finallywith brine (5mL). The organic layers combined dried ($MgSO_4$) concentrated and left over night on the high *vacuo*. The crude product was purified by recrystallization from proper solvent as indicated in Tables 1-2.

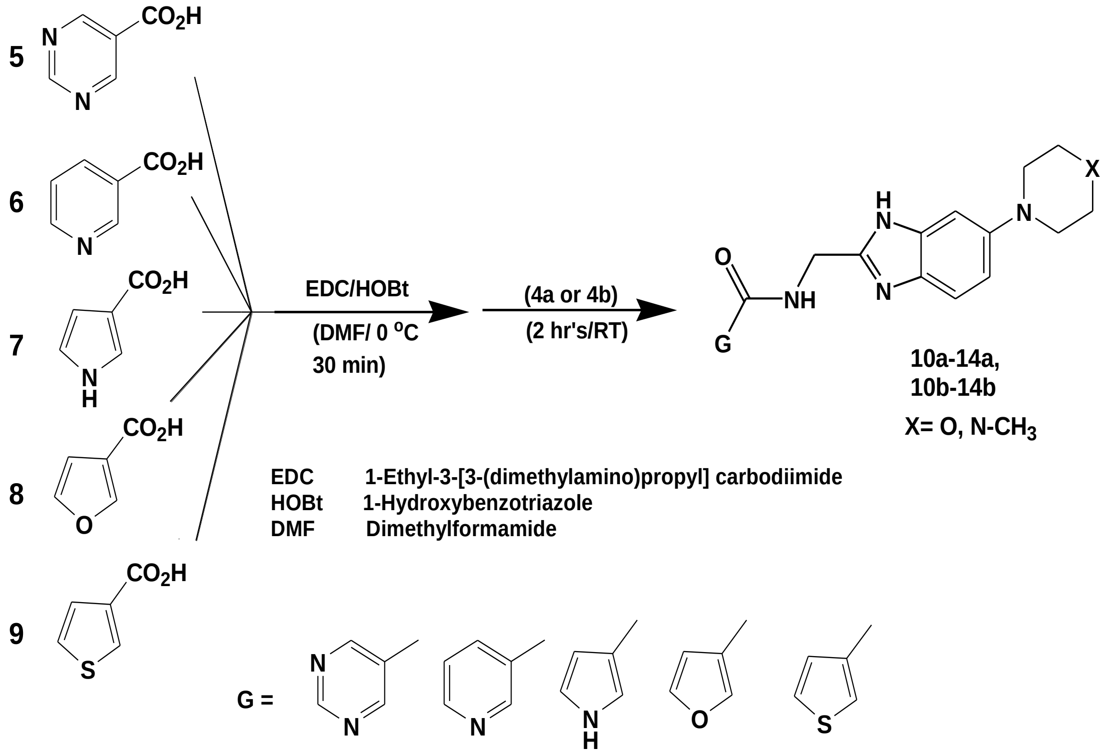


Figure (2):- Coupling of 2-(aminomethyl)-5-morpholiny and 2-(aminomethyl)-5-piperazinyl benzimidazoles (4a and 4b) with hetero carboxylic acid (5-9)

RESULT AND DISCUSSION:

Chemistry

The general synthetic route chosen for such preparation of compounds (10a-14a) and (10b-14b)included four steps (Figures 1-2). As well-known bioheterocyclic systems; both N-methylpiperazine and morpholine separately were treated with 4-fluoro-1,2-dinitrobenzene in the presence of Na_2CO_3/CH_3OH under reflux. This easy nucleophilic aromatic substitution afforded the two dinitrocompounds 2a and 2b which were confirmed by all spectroscopic tools, such as mass spectroscopy, which was consistent with the assigned structure m/z 266 and 253 as the base peak for 2a and 2b respectively. Up on reduction using $SnCl_2$/HCl; both 2a and 2b were converted to the corresponding diamino compounds 3a and 3b; IR showed the N-H stretching at 3322 and 3306 cm^{-1} and ^1H NMR showed appearance of D_2O-exchangeable broad peak in the range of 4.78-5.12 and 5.08-5.27 ppm consistent with the four N-H protons in 3a and 3b respectively. As precursors for benzimidazole synthesis, 3a and 3b were condensed with the carboxylic acid; glycine according to Phillip's method; the 2 and 5 disubstituted benzimidazoles 4a and 4b; 2-(aminomethyl)-5-(4-methylpiperazinyl)benzimidazole and 2-(aminomethyl)-5-(morpholinyl)benzimidazole were formed. The structures of 4a and 4b were confirmed using elemental analysis and spectral data; since^1H-NMR showed theN-Hprotons of (imidazole ring) being detected in the range of (9.99-10.61ppm) as broad peaks. ^{13}C NMR showed the appearance of new methylene carbon at 35.3 and 36.2 ppm The IR stretching has showed C=N as the imino group for the new formed imidazole five memebred ring at 1634 and 1646 $cm^{-1.}$ The N-H of imidazole rings for 4a and 4b at 3421 and 3411 $cm^{-1.}$, while the mass spectral data has been used to cofirm the structure through mass peak for each structure. As the final step; included mixing both the hetercyclic carboxylic acid 5,6,7,8 and 9 separatelty with the well known coupling reagents (EDC and HOBt) in DMF as a solvent in ice bath for 30 minutes followed by adding 4a and 4b seperately under stirring at room temperature. The target structures (10a-14a) and (10b-14b) were confirmed through elemental analysis and spectroscopic tools; since ^1H-NMR has showed all the amide N-H proton as a triplet peak-due to the clear cut evidence arised from coupling with the methylene group - in each compound with chemical shift in the range between (9.00and 9.53 ppm) and with j coupling in the range between (5.8 and 6.2 Hz). Meanwhile the methylene goroup in each compound has been detected as a doublet-due to coupling with amide proton- with the same j coupling mentioned above. The ^{13}C-NMR confirmed the carbonyl streching in the range between 161.1 and 168.9 ppm for all compounds.The IR showed significant streching that confirmed the N-H amide through having broad absorption in the range between 3324 and 3388 cm^{-1} coinciding with indicative peaks towards confirming C=O through streching appearance in the range (1660-1681cm^{-1}). All the compounds were confirmed by elemental analysis as illustrated in Tables 1-2.

Table 1:- Physiochemical parameters of compounds (10a-14a)

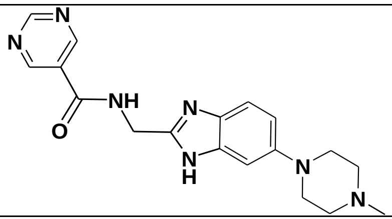
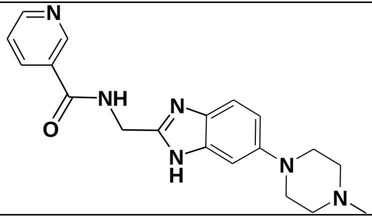
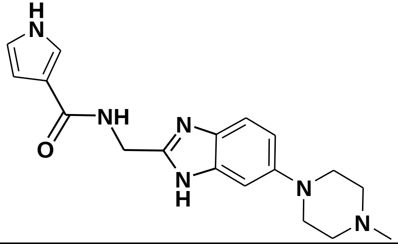
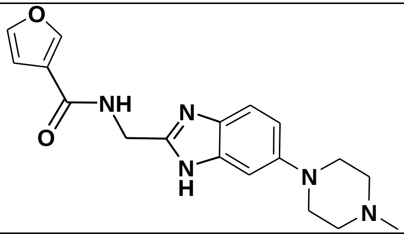
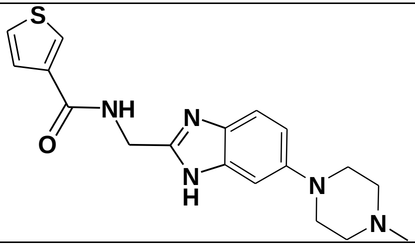
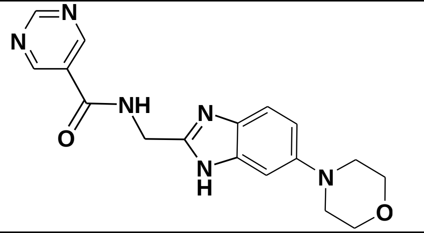
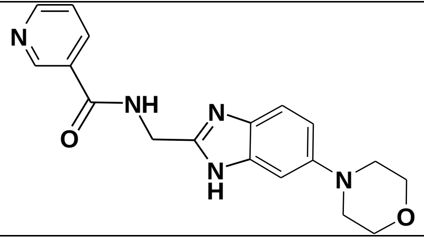
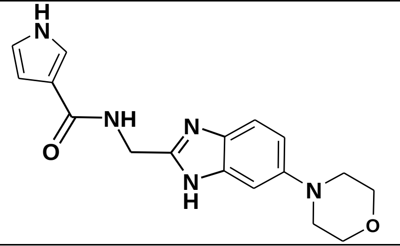
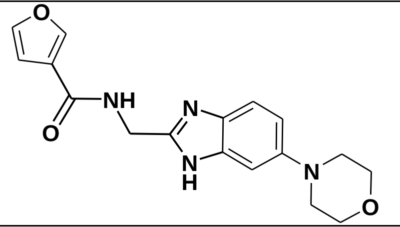
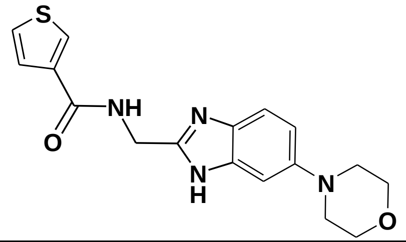
Sr. No.	**Structure**	**Melting point (^{0}C) (Cryst. solvent) and Molecular Formula**	**Yield (%)**	**Elemental Analysis % Calcd./ Found**
10a		247-251 Acetonl/Hexane $C_{18}H_{21}N_7O$	86	C, 61.52/61.45 H, 6.02/6.09 N,27.90/27.96 O, 4.55/4.57
11a		254-255 Acetonl/Hexane $C_{19}H_{22}N_6O$	87	C, 65.12/65.15 H, 6.33/6.38 N,23.98/24.00 O, 4.57/4.59
12a		2234-235 Ethanol $C_{18}H_{22}N_6O$	67	C, 63.89/63.93 H, 6.55/6.58 N, 24.83/24.84 O, 4.73/4.75
13a		210-211 Ethanol $C_{18}H_{21}N_5O_2$	97	C, 63.70/63.73 H, 6.24/6.28 N,20.64/20.67 O, 9.43/9.45
14a		229-230 Ethanol $C_{18}H_{21}N_5OS$	92	C,60.82/60.80 H, 5.95/5.89 N,19.70/19.65 O, 4.50/4.44 S, 9.02/9.07

Table 2:- Physiochemical parameters of compounds (10b-14b)

Sr. No.	**Structure**	**Melting point (^{0}C) (Cryst. solvent) and Molecular Formula**	**Yield (%)**	**Elemental Analysis % Calcd./ Found**

10b		233-235 Acetone/Hexane $C_{17}H_{18}N_6O_2$	91	C, 60.34/60.37 H, 5.36/5.40 N, 24.84/24.89 O, 9.46/9.49
11b		227-228 Acetone/Hexane $C_{18}H_{19}N_5O_2$	88	C, 64.08/64.09 H, 5.68/5.71 N, 20.76/20.79 O, 9.48/9.53
12b		220-221 Ethanol $C_{17}H_{19}N_5O_2$	67	C, 62.75/62.77 H, 5.89/5.94 N, 21.52/21.55 O, 9.83/9.87
13b		234-235 Ethanol $C_{17}H_{18}N_4O_3$	86	C, 62.57/62.57 H, 5.56/5.57 N, 17.17/17.19 O, 14.71/14.73
14b		249-251 Ethanol $C_{17}H_{18}N_4O_2S$	76	C, 59.63/59.65 H, 5.30/5.33 N, 16.36/16.38 O, 9.35/9.36 S, 9.36/9.38

Synthesis of 1-(3,4-Dinitro-phenyl)-4-methyl-piperazine[2a]

(Yield. 75 %), Mp185-187^{0}C. ^{1}H NMR (400MHz, DMSO) δ 2.43 (s, 3H, CH_3), 2.55 (dd, 4H, *J1* = 1.9, *J2* = 9.1 Hz,$2CH_2$), 3.17(dd, 4H, *J1* = 1.9, *J2* = 9.1 Hz,$2CH_2$), 6.43-7.08 (m, 3H, aromatic benzene). ^{13}C NMR (75MHz, DMSO) δ 40.1, 44.1, 53.5, 110.7, 121.8, 131.9, 137.7, 140.1,143.5. IR υ_{max} cm $^{-1}$777 (N-O),1279 (C-N), 1458 (C=C), 2855 (C-H, aliphatic), 3019 (C-H, aromatic), 3188 (N=O). (HRMS: (ES) m/z $(MH)^+$obsd. 266.1019 calcd.266.1015 for $C_{11}H_{14}N_4O_4$

Synthesis of 4-(3,4-Dinitro-phenyl)-morpholine[2b]

(Yield. 74 %), Mp 190-192 ^{0}C. ^{1}H NMR (400MHz, DMSO) δ 2.66 (dd, 4H, *J1* = 1.9, *J2* = 9.1 Hz,$2CH_2$), 3.33 (dd, 4H, *J1* = 1.9, *J2* = 9.1 Hz,$2CH_2$), 6.83-7.91 (m, 3H, aromatic benzene). ^{13}C NMR (75MHz, DMSO) δ 45.0, 47.9, 115.2, 126.1, 135.8, 142.0, 143.7,149.0. IR υ_{max} cm $^{-1}$765

(N-O),1266 (C-N), 1112 (C-O), 1445 (C=C), 2810(C-H, aliphatic), 3002 (C-H, aromatic), 3179 (N=O). (HRMS: (ES) m/z $(MH)^+$obsd. 253.0701 calcd. 253.0699 for $C_{10}H_{11}N_3O_5$

Synthesis of 4-(4-Methyl-piperazin-1-yl)-benzene-1,2-diamine [3a]

(Yield. 70 %), Mp 198-200 ^{0}C. ^{1}H NMR (400MHz, DMSO) δ 2.22 (s, 3H, CH_3), 2.31 (dd, 4H, *J1* = 1.9, *J2* = 9.1 Hz,2CH_2), 3.09 (dd, 4H, *J1* = 1.9, *J2* = 9.1 Hz,2CH_2), 4.78-5.12 (br,4H, N-H), 6.23-7.65 (m, 3H, aromatic benzene). ^{13}C NMR (75MHz, DMSO) δ 39.1 , 43.7 , 48.1 , 113.2, 124.6, 130.1, 137.6, 142.8,146.1. IR υ_{max} cm $^{-1}$1266 (C-N), 1451 (C=C), 2833 (C-H, aliphatic), 3011 (C-H, aromatic), 3322, (N-H, amine). (HRMS: (ES) m/z $(MH)^+$obsd. 206.1536 calcd. Exact Mass: 206.1531for $C_{11}H_{18}N_4$

Synthesis of 4-Morpholin-4-yl-benzene-1,2-diamine [3b]

(Yield.71 %), Mp 201-203 ^{0}C. ^{1}H NMR (400MHz, DMSO) δ 2.45 (dd, 4H, *J1* = 1.9, *J2* = 9.1 Hz,2CH_2), 3.22 (dd, 4H, *J1* = 1.9, *J2* = 9.1 Hz,2CH_2), 5.08-5.27 (br,4H, N-H), 6.25-7.73 (m, 3H, aromatic benzene). ^{13}C NMR (75MHz, DMSO) δ 42.1.0 , 44.9 , 111.2, 126.1, 135.8, 142.0, 143.7,149.0. IR υ_{max} cm $^{-1}$1275 (C-N), 1100 (C-O), 1434 (C=C), 2811(C-H, aliphatic), 3000 (C-H, aromatic), 3306 (N-H). (HRMS: (ES) m/z $(MH)^+$obsd. 193.1220 calcd. 193.1215 for $C_{10}H_{15}N_3O$.

Synthesis of 5-(4-Methyl-piperazin-1-yl)-1H-benzoimidazol-2-yl]-methylamine[4a]

(Yield.79 %), Mp 217-219 ^{0}C. ^{1}H NMR (400MHz, DMSO) δ 2.61 (dd, 4H, *J1* = 1.9, *J2* = 9.1 Hz,2CH_2), 3.34 (dd, 4H, *J1* = 1.9, *J2* = 9.1 Hz,2CH_2), 4.78 (s, 2H, CH_2), 6.56-7.19 (m, 3H, aromatic benzene), 9.99-10.61 (br, NH). ^{13}C NMR (75MHz, DMSO) δ 35.3 , 43.7, 45.6 , 47.9,119.1, 128.2, 135.3, 144.0, 145.9, 153.2, 157.2, IR υ_{max} cm $^{-1}$1246 (C-N), 1411 (C=C), 1646 (C=N), 2839 (C-H, aliphatic), 3077 (C-H, aromatic), 3421 (N-H, amine). HRMS: (ES) m/z $(MH)^+$obsd. : 245.1645 calcd. : 245.1640 for $C_{13}H_{19}N_5$

Synthesis of 5-Morpholin-4-yl-1H-benzoimidazol-2-yl)-methylamine[4b]

(Yield.73 %), Mp 222-223 ^{0}C. ^{1}H NMR (400MHz, DMSO) δ 2.73 (dd, 4H, *J1* = 1.9, *J2* = 9.1 Hz,2CH_2), 3.55 (dd, 4H, *J1* = 1.9, *J2* = 9.1 Hz,2CH_2), 4.96 (s, 2H, CH_2),5.10-5.56(br, NH_2, amine) (6.85-7.32 (m, 3H, aromatic benzene), 10.09-10.23 (br, NH, imidazole). ^{13}C NMR (75MHz, DMSO) δ 36.2, 44.1, 47.8 , 122.3, 125.2, 137.1, 145.8, 148.1, 153.2, 159.1, IR υ_{max} cm $^{-1}$1055 (C-O), 1222 (C-N), 1400 (C=C), 1634 (C=N), 2827 (C-H, aliphatic), 3065 (C-H, aromatic), 3411 (N-H, amine). HRMS: (ES) m/z $(MH)^+$obsd. 232.1329 calcd. 232.1324 for $C_{12}H_{16}N_4O$.

Synthesis of Pyrimidine-5-carboxylic acid [6-(4-methyl-piperazin-1-yl)-1H-benzoimidazol-2-ylmethyl]-amide [10a]

^{1}H NMR (400MHz, DMSO) δ 2.59 (s, 3H,CH_3), 2.68 (dd, 4H, *J1* = 2.1, *J2* = 9.6 Hz,2CH_2), 3.13 (dd, 4H, *J1* = 2.1, *J2* = 9.6 Hz,2CH_2), 4.13 (d, 2H, *J* = 6.02Hz, CH2), 6.60-7.66 (m, 3H, aromatic benzene), 9.11-9.41 (br, 3H, aromatic pyrimidine), 9.53 (t, NH, *J* =6.01 Hz ,amide), 9.88-10.13 (b, NH, imidazole). ^{13}C NMR (75MHz, DMSO) δ 36.8 , 40.0 , 44.1, 55.6 , 102.3, 100.9, 115.5 , 128.1 , 129.6 , 132.3 , 137.9 , 142.3 , 149.6, 153.6 , 166.2. IR υ_{max} cm $^{-1}$ 1288 (C-N), 1114 (C-O), 1437 (C=C), 1606 (C=N), 1681 (C=O), 2836 (C-H, aliphatic), 3033 (C-H, aromatic), 3354 (N-H, amide), 3422 (N-H, amine). (HRMS: (ES) m/z $(MH)^+$obsd. 351.1799 calcd.351.1808 for $C_{18}H_{21}N_7O$.

Synthesis of N-[6-(4-Methyl-piperazin-1-yl)-1H-benzoimidazol-2-ylmethyl]-nicotinamide [11a]

^{1}H NMR (400MHz, DMSO) δ 2.47 (s, 3H, CH_3), 2.58 (dd, 4H, *J1* = 1.9, *J2* = 9.2 Hz,2CH_2), 3.07 (dd, 4H, *J1* = 1.9, *J2* = 9.2 Hz,2CH_2), 4.08 (d, 2H, *J* = 6.11Hz, CH_2), 6.51-7.43 (m, 3H, aromatic benzene), 8.78-9.01 (m, 4H, aromatic pyridine), 9.11 (t, NH, *J* = 6.11 Hz ,amide), 9.71-10.13 (br, NH, imidazole). ^{13}C NMR (75MHz, DMSO) δ 38.2, 42.0, 46.3, 56.1, 108.4, 103.8, 119.5, 129.3, 131.1, 137.8, 145.3, 151.6, 157.3, 164.3. IR υ_{max} cm $^{-1}$ 1278 (C-N), 1112 (C-O), 1445 (C=C), 1613 (C=N), 1679 (C=O), 2833 (C-H, aliphatic), 3011 (C-H, aromatic), 3375 (N-H, amide), 3426 (N-H, amine). (HRMS: (ES) m/z $(MH)^+$ obsd. 350.1823 calcd.350.1855for $C_{19}H_{22}N_6O$.

Synthesis of 1H-Pyrrole-3-carboxylic acid [6-(4-methyl-piperazin-1-yl)-1H-benzoimidazol-2-ylmethyl]-amide[12a]

^{1}H NMR (400MHz, DMSO) δ 2.51 (s, 3H,CH_3), 2.61 (dd, 4H, *J1* = 2.09, *J2* = 9.5 Hz,2CH_2), 3.17 (dd, 4H, *J1* = 2.1, *J2* = 9.6 Hz,2CH_2), 4.24 (d, 2H, *J* = 6.2Hz, CH2), 6.66-7.61 (m, 3H, aromatic benzene), 8.91-9.13 (m, 3H, pyrrolyl), 9.48 (t, NH, *J* =6.2 Hz ,amide), 9.98-11.81 (br, NH, imidazole and pyrrole). ^{13}C NMR (75MHz, DMSO) δ 36.2 , 41.0 , 45.6 , 55.5 , 107.5, 101.6 ,120.1, 125.4 , 131.1 ,137.8, 133.9 , 135.3, 150.0, 152.1 , 167.8. IR υ_{max} cm $^{-1}$1278 (C-N), 1112 (C-O), 1445 (C=C), 1600 (C=N), 1679 (C=O), 2833 (C-H, aliphatic), 3011 (C-H, aromatic), 3370 (N-H, amide), 3401 (N-H, amine). (HRMS: (ES) m/z $(MH)^+$ obsd. 338.1842 calcd.338.1855 for $C_{18}H_{22}N_6O$.

Synthesis of Furyl-3-carboxylic acid [6-(4-methyl-piperazin-1-yl)-1H-benzoimidazol-2-ylmethyl]-amide [13a]

^{1}H NMR (400MHz, DMSO) δ 2.56 (s, 3H,CH_3), 2.69 (dd, 4H, *J1* = 1.9, *J2* = 9.6 Hz,2CH_2), 3.19 (dd, 4H, *J1* = 1.9, *J2* = 9.6 Hz,2CH_2), 4.31 (d, 2H, *J* = 6.0Hz, CH_2), 6.40-7.39 (m, 3H, aromatic benzene), 8.71-8.99 (m, 3H, Furyl), 9.11 (t, NH, *J* =6.0 Hz ,amide), 9.98-12.21 (br, NH, imidazole). ^{13}C NMR (75MHz, DMSO) δ 39.1 , 44.3 , 48.1 , 59.4 , 103.3 , 105.2 , 112.7 , 133.7 , 135.0 ,138.6 , 139.4 , 149.8 , 150.5, 154.4 , 165.2. IR υ_{max} cm $^{-1}$ 1278 (C-N), 1112 (C-O), 1445 (C=C), 1592 (C=N), 1671 (C=O), 2833 (C-H, aliphatic), 3011 (C-H, aromatic), 3380 (N-H, amide), 3425 (N-H, amine). (HRMS: (ES) m/z (MH)$^{+}$ obsd. 339. 1692 calcd. 339. 1695 for $C_{18}H_{21}N_5O_2$.

Synthesis of Thienyle-3-carboxylic acid [6-(4-methyl-piperazin-1-yl)-1H-benzoimidazol-2-ylmethyl]-amide [14a]

^{1}H NMR (400MHz, DMSO) δ 2.39 (s, 3H,CH_3), 2.57 (dd, 4H, *J1* = 1.8, *J2* = 9.3 Hz,2CH_2), 2.89 (dd, 4H, *J1* = 1.8, *J2* = 9.3 Hz,2CH_2), 3.99 (d, 2H, *J* = 6.1Hz, CH_2), 6.31-7.19 (m, 3H, aromatic benzene), 8.64-8.91 (m, 3H, thienyl), 9.00 (t, NH, *J* =6.1Hz,amide), 9.71-10.11 (br, NH, imidazole. ^{13}C NMR (75MHz, DMSO) δ 37.7, 41.6 , 44.5 , 53.2 , 100.1 , 103.8 , 111.4 , 127.4 , 131.1 ,133.3 , 136.7 , 141.0 , 145.6, 155.3, 163.0. IR υ_{max} cm $^{-1}$ 1275 (C-N), 1123 (C-O), 1453 (C=C), 1612(C=N), 1672 (C=O), 2821 (C-H, aliphatic), 3021 (C-H, aromatic), 3388 (N-H, amide), 3422 (N-H, amine). (HRMS: (ES) m/z (MH)$^{+}$ obsd. 355.1461 calcd.355.1467 for $C_{18}H_{21}N_5OS$.

Synthesis of Pyrimidine-5-carboxylic acid (6-morpholin-4-yl-1H-benzoimidazol-2-ylmethyl)-amide [10b]

^{1}H NMR (400MHz, DMSO) δ 2.91 (dd, 4H, *J1* = 2.0, *J2* = 10 Hz,2CH_2), 3.33 (dd, 4H, *J1* = 2.0, *J2* = 10 Hz,2CH_2), 4.11 (d, 2H, *J* = 5.8 Hz, CH_2), 6.65-7.76 (m, 3H, aromatic benzene),8.88-9.00 (m, 3H, pyrimidine), 9.51 (t, NH, *J =5.8 Hz* ,amide), 10.44-12.11 (br, NH, imidazole). ^{13}C NMR (75MHz, DMSO) δ 40.8 , 49.3 , 50.4 , 109.0 , 111.4 , 119.8 , 138.9 , 141.2 ,141.9 , 144.7 , 149.8 , 155.1, 157.4 , 165.9. IR υ_{max} cm $^{-1}$1278 (C-N), 1127(C-O), 1453(C=C), 1611 (C=N), 1671 (C=O), 2843 (C-H, aliphatic), 3015 (C-H, aromatic), 3366 (N-H, amide), 3419 (N-H, amine). (HRMS: (ES) m/z (MH)$^{+}$ obsd. 338.1499 calcd.338.1491 for $C_{17}H_{18}N_6O_2$.

Synthesis of N-(6-Morpholin-4-yl-1H-benzoimidazol-2-ylmethyl)-nicotinamide [11b]

^{1}H NMR (400MHz, DMSO) δ 2.78 (dd, 4H, *J1* = 1.9, *J2* = 9.6 Hz,2CH_2), 3.34 (dd, 4H, *J1* = 1.9, *J2* = 9.6 Hz,2CH_2), 4.19 (d, 2H, *J* = 6.0Hz, CH_2), 6.53-7.55 (m, 3H, aromatic benzene), 9.00-9.32 (m, 3H, pyridine), 9.26 (t, NH, *J* =6.0Hz ,amide), 9.76-11.78 (br, NH, imidazole). ^{13}C NMR (75MHz, DMSO) δ 41.1, 45.2 , 50.2 , 101.2 , 109.8 , 115.8 , 139.7 , 140.7 , 144.2.6 , 147.3 , 149.8 , 150.5, 154.3 , 163.9. IR υ_{max} cm $^{-1}$ 1281 (C-N), 1112 (C-O), 1445 (C=C), 1600 (C=N), 1660 (C=O), 2833 (C-H, aliphatic), 3011 (C-H, aromatic), 3363 (N-H, amide), 3400 (N-H, amine). (HRMS: (ES) m/z (MH)$^{+}$ obsd. 337.1534 calcd.337.1539 for $C_{18}H_{19}N_5O_2$.

Synthesis of 1H-Pyrrole-3-carboxylic acid (6-morpholin-4-yl-1H-benzoimidazol-2-ylmethyl)-amide [12b]

^{1}H NMR (400MHz, DMSO) δ 2.69 (dd, 4H, *J1* = 1.9, *J2* = 9.6 Hz,2CH$_2$), 3.19 (dd, 4H, *J1* = 1.9, *J2* = 9.6 Hz,2CH$_2$), 4.31 (d, 2H, *J* = 6.2 Hz, CH$_2$), 6.23-7.12 (m, 3H, aromatic benzene), 8.66-8.71 (m, 3H, pyrrolyl), 9.31 (t, NH, *J* =6.2Hz ,amide), 10.11-11.34 (br, 2NH, imidazole and pyrrole. ^{13}C NMR (75MHz, DMSO) δ 39.3, 44.3, 48.1, 103.3, 105.2, 112.7, 133.7, 135.0, 138.6, 139.4, 149.8, 150.5, 154.4, 166.6. IR υ_{max} cm $^{-1}$1278 (C-N), 1112 (C-O), 1445 (C=C), 1608 (C=N), 1667 (C=O), 2833 (C-H, aliphatic), 3011 (C-H, aromatic), 3385 (N-H, amide), 3437 (N-H, amine). (HRMS: (ES) m/z (MH)$^+$ obsd. 325.1533 calcd.325.1539for $C_{17}H_{19}N_5O_2$.

Synthesis of Furyl-3-carboxylic acid (6-morpholin-4-yl-1H-benzoimidazol-2-ylmethyl)-amide [13b]

^{1}H NMR (400MHz, DMSO) δ 2.65 (dd, 4H, *J1* = 1.8, *J2* = 9.4 Hz,2CH$_2$), 3.24 (dd, 4H, *J1* = 1.8, *J2* = 9.4 Hz,2CH$_2$), 4.44 (d, 2H, *J* = 6.0Hz, CH$_2$), 6.89-7.88 (m, 3H, aromatic benzene), 8.41-8.79 (m, 3H,Furyl), 9.25 (t, NH, *J* =6.0 Hz ,amide), 9.75-12.34 (br, NH, imidazole. ^{13}C NMR (75MHz, DMSO) δ 40.8, 44.1, 46.2, 100.1, 110.5, 114.8, 123.1, 133.5,139.0, 140.8, 142.9, 157.0, 158.2, 168.9.IR υ_{max} cm^{-1}1254 (C-N), 1156 (C-O), 1411(C=C), 1617 (C=N), 1669(C=O), 2833(C-H, aliphatic), 2998(C-H, aromatic), 3324(N-H, amide), 3444 (N-H, amine). (HRMS: (ES) m/z (MH)$^+$ obsd. 326.1374 calcd.326.1379 for $C_{17}H_{18}N_4O_3$.

Synthesis of Thienyle-3-carboxylic acid (6-morpholin-4-yl-1H-benzoimidazol-2-ylmethyl)-amide [14b]

^{1}H NMR (400MHz, DMSO) δ 2.45 (dd, 4H, *J1* = 1.9, *J2* = 9.6 Hz,2CH$_2$), 3.06 (dd, 4H, *J1* = 1.9, *J2* = 9.6 Hz,2CH$_2$), 4.19 (d, 2H, *J* = 6.0Hz, CH$_2$), 6.26-7.28 (m, 3H, aromatic benzene), 8.41-8.79 (m, 3H, thienyl), 9.08 (t, NH, *J* =6.0 Hz ,amide), 9.37-11.21 (br, NH, imidazole). ^{13}C NMR (75MHz, DMSO) δ 38.2 , 42.3 , 47.1 , 100.3 , 103.9 , 110.7 , 131.7 , 132.3 , 135.6 , 136.1 , 142.9 , 152.8, 158.2 , 161.1. IR υ_{max} cm $^{-1}$ 1266 (C-N), 1133 (C-O), 1431(C=C), 1609 (C=N), 1659(C=O), 2812(C-H, aliphatic), 3001(C-H, aromatic), 3367 (N-H, amide), 3419 (N-H, amine). (HRMS: (ES) m/z (MH)$^+$ obsd.342.1145 calcd.342.1150 for $C_{17}H_{18}N_4O_2S$.

Biology

All the compounds synthesized in this work were examined for their in vitro antimicrobial activities against Gram-negative bacteria [*Escherichia coli (E. coli*)], and Gram-positive[*Staphylococcus aureus*(*S. aureus*)]and the yeasts like fungi (*C.albicans and A.niger*). Compared to the reference substances; Ciprofloxacin and Fluconazole, all the synthesized compounds were screened for antibacterial and antifungal activities against studied strains by cup-plate agar diffusion method in nutrient agar medium with an incubation of 24 h at 37^0C. The zone of inhibition was measured in millimeters using 12.5, 25, 37.5, and 50 ppm concentrations of the synthesized compounds. Ciprofloxacin was used as reference and DMF as a solvent and a control.

Antibacterial screening results (the zone of inhibition) illustrated in **Table 3**, revealed that most synthesizedcompounds tested showed significant degree of antibacterial activity. The compounds exhibited zone of inhibition of 12-26 mm in diameter where as standard, Ciprofloxacin showed a zone of inhibition of 26 and 25 mm in diameter against *S.aureus*and *E.coli*at 4 ppm concentration respectively. Among the synthesized compounds, it was observed thatthienyl-containing compounds (14a and 14b) have showed a remarkable antibacterial effect compared to furyl, pyrrolyl, pyridyl, pyrimidinyl-containing compounds in both morpholine and N-methylpeprazine systems; as a result the two compounds 14a and 14b showed the highest antibacterial activity and they showed an equipotent antimicrobial activity of Ciprofloxacin and they are indeed the most profitable against both strains. Compounds 12b showed the lowest activity against both strains.

Table (3):- Antibacterial activity data of all synthesized compounds

Compound No	**Zone of inhibition(mm)**							
	S.aureus **Concentration(ppm)**				***E.coli*** **Concentration(ppm)**			
	12.5	**25**	**37.5**	**50**	**12.5**	**25**	**37.5**	**50**
Ciprofloxacin	26				25			
10a	20	22	23	24	19	21	23	24
11a	19	21	22	23	20	22	23	24
12a	16	17	18	19	14	15	16	17
13a	16	17	19	21	14	15	17	19
14a	21	23	24	26	21	23	24	25
10b	21	22	23	24	16	18	21	24
11b	16	17	18	20	14	16	18	20
12b	12	14	15	16	12	13	14	15
13b	17	18	19	20	17	18	19	21
14b	21	23	25	26	20	23	24	25

The results of antifungal activity of the tested compounds (10a-14a) and (10b-14b) were found to be quite different from their antibacterial activity. Sensitivity of the selected fungal pathogens to all synthetic compounds was determined in vitro at four concentrations (12.5, 25, 37.5 and 50 ppm). As general the synthetic compounds exhibited zone of inhibition of 12-27 mm in diameter whereas standard, Fluconazole showed a zone of inhibition of 27 and 26 mm in diameter against *C. albicans*and *A. niger*at 4 ppm concentration respectively. The antifungal screening results of the synthesized compounds were showed that the compounds; 13a and 13b showed the same antifungal activity when compared with Fluconazole. Compounds 12b was found to be least active against both the strains among the tested compounds, compound 13b showed better activity against both the strains. It is an obvious that the presence of furylgroup in addition to the morpholinyl group in the same compound provided a clear and significant antifungal activity while the presence of thienylin addition to the piperazinyl substituents in the same compound provided a clear and significant antibacterial activity.

Table 4. Antifungal activity data of the synthesized compounds

Compound No	**Zone of inhibition(mm)**							
	C.albicans				***A.niger***			
	Concentration (ppm)				**Concentration (ppm)**			
	12.5	**25**	**37.5**	**50**	**12.5**	**25**	**37.5**	**50**
Fluconazole	**27**				**26**			
10a	15	16	17	20	18	21	23	25
11a	14	17	18	20	18	19	21	23
12a	12	14	16	17	12	15	17	19
13a	21	23	25	27	21	23	24	25
14a	19	20	23	25	19	20	23	24
10b	18	19	21	23	16	18	21	24
11b	17	19	22	23	14	16	22	23
12b	12	14	15	16	12	13	14	15
13b	21	23	25	27	21	23	24	26
14b	16	18	19	20	15	17	19	21

CONCLUSION:

In conclusion, a series of benzimidazole derivatives have been synthesized successfully in appreciable yields, cheap and convenient ways and screened for their in vitro antimicrobial activity. From the antibacterial activity study, it was observed that compounds 14a and 14b showed the best activity. Thus it was concluded that among all benzimidazole derivatives, antibacterial activity increases by the synchronizing existence of both thienyl and piperazinyl substituents in the same compound, this activity is as equipotent as Ciprofloxacin. From antifungal activity study, it was observed that compound 13b showed better activity against both the strains whereas compounds 12 b was found to be the least active. Thus it was concluded that the compounds having furyl and morpholinyl substituent's synchronizingly is the most potent antifungal with an equipotent feature to Fluconazole. However, In-vivo future pharmaceutical investigations and studies might lead to grab the attention towards compounds (14a and 14b) and (13a and 13b) as promising antibacterial and antifungal drugs respectively that could be the drugs of choice for some bacterial and fungal infections in the future.

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