Synthesis, spectroscopic characterization, and antibacterial evaluation of new Schiff bases bearing benzimidazole moiety

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Abstract. The present work comprise synthesis of new derivatives for Schiff bases bearing benzimidazole ring. Compounds 1(a-d) were prepared by reaction of o-phenylenediamine with various of amino acids (glycine, alanine, phenyl alanine and tyrosine) in the presence 6N HCl to yielded derivatives of benzimidazole compounds containing free –NH₂ group. Then these compounds used to prepare different Schiff bases through reaction with various of aromatic aldehydes. The chemical structure of synthesized compounds were confirmed by FTIR,¹H,¹³C-NMR, and ¹³C-NMR dept135 spectroscopy. Some selected compounds were evaluated in vitro for their antibacterial activity against two types of Gram-positive bacteria namely (Staphylococcus aureus, Bacillus subtilis) and Gram-negative bacteria namely (Pseudomonas aeruginosa, Escherichia coli). Most of the results of the antibacterial activity of these compounds were good when compared with the standard antibiotic ampicillin and ciprofloxacin.

1. Introduction

Due to their remarkable biological activity and diverse clinical application, Benzimidazole derivatives occupied a major part in the field of pharmaceutical chemistry and very important field of heterocyclic compounds. The benzimidazole nucleus moiety is existing in many Benzimidazole compounds are consider a promising field of bioactive heterocyclic compounds. Specifically this nucleus is constitution of vitamin-B12 [9]. Because of the immense importance and versatile biological activities displayed by benzimidazole, Attempts have been made from time to time to create libraries of these compounds and evaluation them for biological activities [10]. Schiff base hold a major part in the field of pharmaceutical research due to their high biological activity. Thus, numerous attempts to develop and design of new Schiff base still arouse interest of pharmaceutical and medicinal researchers [11].

Schiff bases which have a various of heterocyclic ring were reported to posses a wide spectrum of biological activities including antiviral [12], anticancer [13], cytotoxic [14], antimicrobial [15], antibacterial [16-17], anticonvulsant [18]. In coordination chemistry of transition metals, metal complexes which derivative from Schiff bases have been usually utilized as chelating ligands in radiopharmaceutical for cancer attack and agrochemicals such as pesticide. Therefore, they constitute a fascinating field of chelating agents effective of coordination with metals ion which use as style for biological systems [19-21].
2. Experimental Section
2.1 General
Melting points were taken in an electrically heated using Stuart SMP3 instrument and are uncorrected. FT-IR spectra were recorded on (Shimadzu FT-IR- 8400S spectrophotometer at the Chemistry department/ College of education for pure science/ University of Diyala) by using KBr disc(v,cm⁻¹). The ¹H and ¹³C-NMR spectra were recorded on (Bruker 400MHz at the Jordan University for science and technology /Jordan ) by using tetramethylsilane (TMS) as an internal standard and DMSO-d₆ as solvent. The purity of the compounds was checked by TLC on silica gel plates using ultraviolet lamp(365nm and 254nm).

2.2 General procedure for synthesis of compounds 1(a–d) [22]: A solution of o-phenylenediamine (6.5g, 0.06 mol) and amino acids (glycine, alanine, phenylalanine and tyrosine) (0.12 mol) in 6N hydrochloric acid (20 ml) was heated to reflux with stirring for 6h. The progress of the reaction was monitored by TLC plate. On completion of the reaction, the reaction mixture was cooled to room temperature and the pH was adjusted to 7.2 using 1N sodium hydroxide solution to obtain buff colored product. The product was recrystallized using ethanol as solvent.

(IH-benzo[d]imidazol-2-yl)methanamine (1a): White crystals, yield 66%. m.p: 257 – 259°C, IR₉max (KBr/cm⁻¹): NH₂ (3387, 3363), N-H benzimidazole (3288), aromatic C-H (3028), aliphatic C-H (2864, 2754), C=N (1633), aromatic C=C (1458, 1593). ¹H –NMR (400MHz, DMSO – d₆) δ : 3.01 (2H, s, CH₂), 4.38 (1H, s, N-H), 8.58 (2H, s, NH₂), 6.3-6.5 (4H,m,Ar – H). ¹³C-NMR (400MHz, DMSO) δ : 44.6 (CH₂), 142.3 (C=N of benzimidazole), 114.7, 117.3, 134.8. ¹³C-Dept 135 NMR (400MHz, DMSO – d₆) δ : 44.4 (CH₂).

1-(IH-benzo[d]imidazol-2-yl)ethanamine (1b): Brown, yield 97%. m.p : 236 – 238°C, IR₉max (KBr/cm⁻¹): NH₂ (3387, 3336), N-H benzimidazole (3286), aromatic C-H (3037,3028), aliphatic C-H (2944, 2856), C=N (1633), aromatic C=C (1458-1593).¹H –NMR (400MHz, DMSO – d₆) δ : 1.2 (3H, d, CH₃), 3.7 (1H, q, C-H), 5.9 (1H, s, N-H benzimidazole), 8.44 (2H, s, NH₂), 6.54-7.81 (4H,m,Ar – H). ¹³C-NMR (400MHz, DMSO) δ : 23.9 (CH₃), 54.3 (CH), 140.9 (C=N of benzimidazole), 140.1, 138.1, 127.1, 119.2,114.9.

(1H-benzo[d]imidazol-2-yl)(phenyl)methanamine (1c): Off White, yield 98% . m.p : 255 – 257°C, IR₉max (KBr/cm⁻¹): N-H benzimidazole (3125), aromatic C-H (3064,3034), aliphatic C-H (2960, 2868), C=N (1625), aromatic C=C (1454-1571).

4-(2-amin o-2-(1H-benzo[d]imidazol-2-yl)ethyl)phenol (1d):Dark brown, yield 77% . m.p : 276–278°C, IR₉max (KBr/cm⁻¹): NH₂ (3385,3363), N-H benzimidazole (3205) overlap with OH , aromatic C-H (3024), aliphatic C-H (2962, 2885), C=N (1608).aromatic C=C (1456-1591).

2.3 General procedure for the synthesis of compounds 2(a–l) [23]: Compounds 1(a–d) (0.01 mol) were add to a solution of the different substituted benzaldehydes (p-bromobenzaldehyde, p-nitro benzaldehyde, p-hydroxybenzaldehyde) (0.012 mol) in dry ethanol 40 ml in RBF. Two drops of glacial acetic acid were also added to the above mixture. The mixture was refluxed for 8-12h and at the end of the reaction; solvents were partially evaporated then poured in to water. The precipitates
were collected by filtration, washed with ether, dried and compounds 2(a-l) were synthesized and recrystallized from the appropriate solvent like ethanol or ethanol-water.

1-(1H-benzo[d]imidazol-2-yl)-N-(4-bromobenzylidene)ethanamine (2a): Yellow, yield 87%. m.p : 287-289°C, IR \( \nu_{\text{max}} \) (KBr/cm\(^{-1}\)) : N-H benzimidazole(3321), aromatic C-H (3030,3055), aliphatic C-H (2997, 2875), C=N (1610), aromatic C=C (1448-1531), C-Br (744).

4-(((1H-benzo[d]imidazol-2-yl)methyl)imino)methyl)phenol (2b): Brown crystals, yield 93%. m.p : 285-287°C, IR \( \nu_{\text{max}} \) (KBr/cm\(^{-1}\)) : N-H benzimidazole and O-H(3253)over lab, aromatic C-H (3088,3032), aliphatic C-H (2943, 2885), C=N (1610), aromatic C=C (1444-1514), \(^1\)H –NMR (400MHz, DMSO – d\(_6\)) \( \delta \) : 3.37 (2H, s, CH\(_2\)), 5.40 (1H, s, N-H), 9.97 (1H, s, OH), 9.41 (1H, s, CH=IN), 6.64-7.65 (4H, m, Ar – H). \(^1\)C –NMR (400MHz, DMSO) \( \delta \) : 47.4 (CH\(_3\)), 142.7 (C=N of benzimidazole), 156.5 (1H,s,C-CH), 158.7(1H,s,CH=CH\(_2\)), 115.3 , 118.7 ,122.03, 127.4 130.5 , 136.19 \(^{13}\)C-Dept 135 NMR (400MHz, DMSO – d\(_6\)) \( \delta \) : 46.8 (CH\(_2\)).

1-(1H-benzo[d]imidazol-2-yl)-N-(4-nitrobenzylidene)ethanamine (2c): Yellow crystals, yield 91%. m.p : 293-295 °C, IR \( \nu_{\text{max}} \) (KBr/cm\(^{-1}\)) : N-H benzimidazole(3321), aromatic C-H (3035,3035), aliphatic C-H (2954, 2850), C=N (1604), aromatic C=C (1445,1535), NO\(_2\)(1340,1516).

1-(1H-benzo[d]imidazol-2-yl)-N-(4-bromobenzylidene)ethanamine (2d): Brown crystals, yield 87%. m.p : 283-285°C, IR \( \nu_{\text{max}} \) (KBr/cm\(^{-1}\)) : N-H benzimidazole(3321), aromatic C-H (3089,3055), aliphatic C-H (2983, 2879), C=N (1613), C=Br (765), aromatic C=C (1466,1536), \(^1\)H –NMR (400MHz, DMSO – d\(_6\)) \( \delta \) : 1.56 (3H, s, CH\(_3\)), 5.70 (1H, s, N-H), 3.97 (1H, s, CH), 9.11 (1H, s, CH=IN), 6.94-7.8 (8H, m, Ar – H). \(^1\)C –NMR (400MHz, DMSO) \( \delta \) : 17.4 (CH\(_3\)), 60.6 (CH), 145.7 (C=N of benzimidazole), 160.7(1H,s,CH=CH\(_2\)),117.3 , 118.7 ,123.03, 130.4, 138.5 , 140.19.

4-(((1H-benzo[d]imidazol-2-yl)ethyl)imino)methyl)phenol (2e): Yellow crystals, yield 91%. m.p : 285 – 287°C, IR \( \nu_{\text{max}} \) (KBr/cm\(^{-1}\)) : N-Hbenzimidazole(3265), O-H (3398), aromatic C-H (3095,309), aliphatic C-H (2852 , 2737), C=N (1625), aromatic C=C (1428-1536),

1-(1H-benzo[d]imidazol-2-yl)-N-(4-nitrobenzylidene)ethanamine (2f): Red, yield 86%. m.p : 302 – 304°C, IR \( \nu_{\text{max}} \) (KBr/cm\(^{-1}\)) : N-H benzimidazole(3245), aromatic C-H (3117,3029), aliphatic C-H (2962, 2807), C=N (1629), aromatic C=C (1470-1596), NO\(_2\)(1557,1338).

1-(1H-benzo[d]imidazol-2-yl)-N-(4-bromobenzylidene)-2-phenylethanamine (2g): Yellow crystals, yield 64%. m.p : 274 – 276°C, IR \( \nu_{\text{max}} \) (KBr/cm\(^{-1}\)) : N-H benzimidazole(3317), aromatic C-H (3109,3001),aliphatic C-H (2919, 2828), C=N (1614), aromatic C=C (1466,1547),C-Br (745).

4-(((1-(1H-benzo[d]imidazol-2-yl)-2-phenylethyl)imino)methyl)phenol (2h): Yellow crystals, yield 76%. m.p : 285 – 286°C, IR \( \nu_{\text{max}} \) (KBr/cm\(^{-1}\)) : N-H benzimidazole(3285),O-H (3425), aromatic C-H (3137), aliphatic C-H (2971,2876), C=N (1614), aromatic C=C (1459,1537). \(^1\)H –NMR (400 MHz, DMSO – d\(_6\)) \( \delta \) : 3.23 (2H, d, CH\(_2\)), 4.83 (1H, q, C-H), 5.93 (1H, s, N-H benzimidazole), 9.53 (1H, s, O-H), 8.39 (1H,s,N=C-H), 6.87-8.10 (13H,m,Ar – H). \(^1\)C –NMR (400 MHz, DMSO– d\(_6\)) \( \delta \) : 65.8 (C-H), 46.3 (CH\(_2\)), 159.8 (=C-H), 163.4 (C-O), 138.3 (C=N of benzimidazole), 113.3 , 117.4 , 120.5, 124.9, 128.7, 130.1 , 133.6 , 139.8 , \(^{13}\)C-Dept 135 NMR (400MHz, DMSO – d\(_6\)) \( \delta \) : 46.1(CH\(_2\)).

1-(1H-benzo[d]imidazol-2-yl)-N-(4-nitrobenzylidene)-2-phenylethanamine (2i): Yellow crystals, yield 78%. m.p : 294– 296°C, IR \( \nu_{\text{max}} \) (KBr/cm\(^{-1}\)) : N-H benzimidazole(3273), aromatic C-H (3097,2996), aliphatic C-H (2827, 2799), C=N (1605), aromatic C=C (1467-1553), NO\(_2\)(1530,1344),
4-(2-(1H-benzo[d]imidazol-2-yl)-2-((4-bromobenzylidene)amino)ethyl)phenol (2j):
Brown crystals, yield 83%. m.p : 290 – 292 °C, IR νmax (KBr/cm⁻¹): O-H(3405), N-H benzimidazole(3205), aromatic C-H (3080,3039), aliphatic C-H (2881,2962), C=O (1608), aromatic C=C (1456-1591), C-Br (740).

4-(2-(1H-benzo[d]imidazol-2-yl)-2-((4-hydroxybenzylidene)amino)ethyl)phenol (2k):
Brown crystals, yield 81%. m.p : 304 – 306°C, IR νmax (KBr/cm⁻¹): N-H benzimidazole(3205), aromatic C-H (3039,3024), aliphatic C-H (2962, 2885), C=O (1608), aromatic C=C (1456-1591), O-H (3446).

4-(2-(1H-benzo[d]imidazol-2-yl)-2-((4-nitrobenzylidene)amino)ethyl)phenol (2l):
Red, yield 64%. mp : 316 – 318°C, IR νmax (KBr/cm⁻¹): O-H(3410), N-H benzimidazole(3205), aromatic C-H (3039,3024), aliphatic C-H (2960 , 2897), C=O (1608), aromatic C=C (1456-1591), NO₂ (1456,1591)), ¹H –NMR (400 MHZ, DMSO – d₆) δ :  3.56 (2H, s, CH₂), 5.90 (1H, s, N-H), 4.47 (1H, t, CH),9.01 (1H, s, CH=N), 6.97-7.90 (12H, m, Ar – H). ¹³C –NMR (400 MHZ, DMSO) δ : 47.4 (CH₂), 63.6 (CH), 149.7 (C=O of benzimidazole), 167.7(=CH), 157(C-O) , 153(C-NO₂), 113.3 , 117.7,122.03, 131.4, 137.5 , 140.19 , 143.5 ¹³C-Dept 135 NMR (400 MHZ, DMSO – d₆) δ : 47.2 (CH₂).

Scheme 1. Synthetic route to the synthesized compounds. Reagents and conditions: (a) Corresponding amino acids, 6N HCl, reflux(8-12)hrs; (b) Corresponding aromatic aldehyde, EtOH/2-3 drops of CH₃COOH
In the present work, benzimidazole derivatives containing Schiff base moiety were synthesized according to the reaction scheme (1). (1H-benzo[d]imidazol-2-yl)methanamine (1a), 1-(1H-benzo[d]imidazol-2-yl)ethanamine (1b), (1H-benzo[d]imidazol-2-yl)(phenyl)methanamine (1c) and 4-(2-amino-2-(1H-benzo[d]imidazol-2-yl)ethyl)phenol (1d), (a-d) were prepared by the condensation reaction of the o-phenylenediamine and amino acids (glycine, alanine, phenyl alanine and tyrosine) in the presence of 6N HCl. The IR spectra of these compounds exhibited broad absorption bands, one of which appearing at (3125-3288) was attributed to the N-H imidazole group. And other, observed at (3336-3421) was assigned to NH$_2$ stretching frequency. In $^1$H-NMR spectra of compounds 1(a,d) exhibited two different signals at (δ 8.4-8.5 ppm) which attributed to NH$_2$ protons and (δ 4.3-5.9 ppm) which assigned to N-H imidazole protons. The $^{13}$C-NMR spectra of compounds 1(a,b) exhibited signals at (δ 140.9 – 142.3 ppm) which attributed to the (C=N) group. The Schiff bases compounds 2(a-l) was prepared by the condensation reaction of compounds 1(a-d) with corresponding aromatic aldehyde in the presence of ethanol and few drops of acetic acid. The structure of all compounds 2(a-l) was confirmed by its IR spectra and compounds 2(b,d,h,l) by $^1$H and $^{13}$C-NMR. The IR spectra of these compounds exhibited broad absorption band at (3205-3321)cm$^{-1}$ was attributed to the N-H imidazole group and band at (1604-1629) which assigned to imine group (N=CH). In $^1$H-NMR spectra of compounds 2(b,d,h,l), the presence of proton of N=CH group was confirmed by one proton singlet at (8.3-9.4)ppm, while signal for imidazole protons of NH group can be observed at (5.4-5.9), $^1$H-NMR spectra revealed the multiplet at (6.6-8.1)ppm corresponding to the four aromatic protons. The $^{13}$C-NMR spectra of compounds 2(b,d,h,l) exhibited signals at (δ 158.7–
167.7 ppm) which attributed to the imine group (-N=CH-) and showed signal at about (δ138.3 – 149.7 ppm) related to benzimidazole (-C=N) group. Signals for benzene ring appeared at about δ 113.3 – 143.5 ppm. In 13CNMR, DEPT-135 of compounds(1a,2b,2h and 2l)show negative signals at around (44.4 – 47.2) for CH2 group.

4. Antibacterial activity

The disk diffusion method was used to screened antibacterial activities of the some compounds synthesized herein against different strains of Gram-positive bacteria namely (Staphylococcus aureus, Bacillus subtilis) and Gram-negative bacteria (Pseudomonas aeruginosa, Escherichia coli). The compounds were tested at concentration of (10 mg /ml and 100 mg /ml). The zone of inhibition was measured in millimeters and was compared with reference standard antibiotic namely ampicillin and ciprofloxacin. The test compounds were dissolved in DMSO to obtain solution of different concentration. The results of antibacterial activity of the synthesized compounds are listed in (table 2) which demonstrate that most of compounds displayed significant activities when compared with the standard antibiotic ampicillin and ciprofloxacin. The antibacterial activities of the test compounds are shown briefly below.

1. The compounds (1a, 2a,2f, 2g, 2k) showed high activity against B. subtilis bacteria.
2. The compounds (2a,2f,2g,2k) showed high activity against E. coli bacteria.
3. The compounds (2a,2g,2k) showed high activity against P. aeruginosa bacteria.
4. The compounds (1a,2a,2g,2k) showed good activity against S.aureus bacteria.

Table 2. Antibacterial activity of synthesized compounds

| Comp no. | Concentration (mg / ml) | Zone of inhibition ( in mm) | Gram-positive | Gram-negative |
|----------|-------------------------|----------------------------|---------------|---------------|
|          |                         |                            | S. aureus | B. subtilis | P. aeruginosa | E. coli |
| 1a       | 10                      | 11                         | 10         | 10         | 10          | 13       |
|          | 100                     | 17                         | 19         | 12         | 14          |          |
| 1b       | 10                      | 12                         | 12         | -          | -           |          |
|          | 100                     | 10                         | 13         | 11         | -           |          |
| 2a       | 10                      | 14                         | 22         | 29         | 25          |          |
|          | 100                     | 15                         | 13         | 12         | -           |          |
| 2f       | 10                      | 14                         | 20         | 13         | 23          |          |
|          | 100                     | -                          | 21         | -          | 20          |          |
| 2g       | 10                      | 15                         | 28         | 20         | 22          |          |
|          | 100                     | 15                         | 11         | -          | -           |          |
| 2l       | 10                      | 14                         | 12         | 12         | 13          |          |
|          | 100                     | 12                         | 11         | 15         | 11          |          |
| 2k       | 10                      | 16                         | 24         | 22         | 27          |          |
Conclusion

Series of new Schiff base attached to benzimidazole ring have been synthesized successfully by condensation reaction between (1H-benzo[d]imidazol-2-yl)methanamine, 1-(1H-benzo[d]imidazol-2-yl)ethanamine, (1H-benzo[d]imidazol-2-yl)(phenyl)methanamine and 4-(2-amino-2-(1H-benzo[d]imidazol-2-yl)ethyl)phenol with various of aromatic aldehydes. The compounds (1a, 2a, 2f, 2g, 2k) showed high activity against *B. subtilis* bacteria, compounds (2a, 2f, 2g, 2k) showed high activity against *E. coli* bacteria, compounds (2a, 2g, 2k) showed high activity against *P. aeruginosa* bacteria and compounds (1a, 2a, 2g, 2k) showed good activity against *S. aureus* bacteria.

References

[1] Sawhney S, Vir D, and Kumar P 1989 *Indian. J. Chem.* B 28 574 - 578
[2] Ibrahim E, Omar A and Khalil M 1980 *European J. Pharm. Sci.* 69 1348 - 1350
[3] Fatma N, Murthy P and Kumar S 1988 *Indain J. Pharm. Sci.* 50 265 - 268
[4] Morinaga H, Yanase T and Nomura M 2004 *Endocrinology.* 145 1860 - 1869
[5] Freeman G, Selleseth D and Rideout J 2000 *Nucleosides, Nucleotides Nucleic Acids* 19 155 - 174
[6] Ates-Alagoz, Z, Alp M, Kus, C,Yilidiz S, Buyukbing, E and Goker H 2006 *Arch. Pharm. Chem. Life Sci.* 339
[7] Lazer E, Farina P and Oliver J 1987, *Agents Actions.* 21 2057 - 2059
[8] Jiang S, Meadows S and Anderson S 2002 *Antimicrob. Agents Chemother.* 46 2569 - 2574
[9] Niel M and Smith M, Heckelman 2001 *The Merk Index, 13th ed*. Merk Co. Inc., New Jersey, p.10074
[10] Ansari K and Lal C 2009 *Eur. J Med. Chem.* 44 4028 - 4033
[11] Vigato P and Tamburini S 2004 *Coord. Chem. Rev.* 248 1717-2128
[12] Vicini P, Geronikaki A, Incerti M, Busonera B, Poni G, Cabras C and IaColla P 2003 *Bioorg. Med. Chem.* 11 4785-4789
[13] Sondhi S, Singh N, Kumar A, Lozach O and Meijer L2006 *Bioorg. Med. Chem.* 14 3758-3765
[14] Tarafder M, Kasbollah A, Saravan N, Crouse K, Ali A and Tin O 2002 *J. Biochem. Mol. Biol. Biophys.* 685
[15] Hui-Ming L, Tan S, Li H, Song Y, ZhuH and Tan R 2007 *Eur. J. Med. Chem.* 42 558-564
[16] Cheng K, Zheng Q, Hou J, Zhou Y, liu C, Zhao J and Zhu H 2010 *Bioorg. Med. Chem.* 18 2447-2455
[17] Cheng K, Zheng Q, Qian Y, Shi L, ZhaoJ and Zhu H 2009 *Bioorg. Med. Chem.* 17 7861-7871
[18] Kucukguzel I, Kucukguzel S, Rollas S, Sanis G Ozdemir O, Bayrak I, Altug T and Stables J 2004 *Il Farmaco* 59 839
[19] Liu G, Liao J, Huang S, Shen G and Yu R 2001 *Anal. Sci.* 17 1031-1036
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