Synthesis, characterization and antibacterial evaluation of some novel bis benzimidazole derivatives.

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Abstract. Serious of some novel bis benzimidazole derivatives were designed and synthesized by nucleophilic substitution reaction of 5-(un)substituted-2-chloromethyl-1H-benzimidazole and 5-(un)substituted-2-mercapto-1H-benzimidazole in the presence of sodium in methanol. Spectral methods of analysis (FT-IR, 1H-NMR, and 13C-NMR) were used to confirm the structures of the synthesized compounds. Antibacterial activity were evaluated for most of the target compounds against four strains of bacteria including (E.coli, P. aeruginosa) as gram-negative bacteria and (B. subtilis, S. aureus) as gram-positive bacteria, the tested compounds showed various activity against both gram-negative and gram-positive bacteria used.

Keywords: benzimidazole, bis benzimidazole, antibacterial activity.

Introduction

In the family of heterocyclic compounds, heterocycles containing nitrogen are an significant class of compounds in the medicinal chemistry and also contributed to the society from industrial and biological point which helps to understand life processes. Benzimidazole ring consider an important heterocyclic moiety in the drug discovery, the literature has showed that the substitutions at the positions 2 and 5 of the benzimidazole compounds are important for showing a wide rang of pharmacological activities including antimicrobial, antitumor, analgesic and anti-inflammatory, anti-hypertensive, and antiviral properties. Owing to the massive importance and varied bioactivities exhibited by benzimidazoles, efforts have been made from time to time to create new derivatives of these compounds and screen them for potential biological activities. The optimization of benzimidazole derivatives based on their structures has resulted in many potent drugs that are currently practiced in the market, such as omeprazole, albendazole, mebendazole, etc.
Materials and methods

Melting points were recorded by stuart smp3 electronic apparatus, FT-IR spectra were recorded on shimadzu FT-IR spectrophotometer, $^1$H and $^{13}$C –NMR spectra were recorded on ( brucker 400 MHz) spectrophotometer using (DMSO) as a solvent and TMS as an internal reference. The compounds were evaluated for their purity on silica gel TLC plates and the visualization of spots achieved by UV light.

General method for the Synthesis of 5-(un)substituted-2-(chloromethyl)-1H-benzimidazole 1(a-c) \(^{(14,15)}\)

A mixture of 4-(un)substituted-o-phenylenediamine (0.1 mole) and chloroacetic acid (0.1 mole) was dissolved in ( 50 ml) 4N HCl and refluxed for 4 hours. The completion of the reaction was checked by T.L.C ( mobile phase: hexane: ethyl acetate 1:2). The reaction mixture was then allowed to cool down and neutralized with ammonium hydroxide solution, the precipitate obtained was dried and recrystallized from (methanol/water).

2-(chloromethyl)-1H-benzimidazole (1a):
yellow crystals, m.p: 147-150 °C, IR (KBr, cm$^{-1}$): N-Hstr (3133), aromtic C-Hstr ( 3050), aliphatic C-Hstr (2900, 2846), C=Nstr (1625), aromatic C=Cstr (1520, 1446), C-Clstr (640), Yield: 93%.

2-(chloromethyl)-1H-benzimidazole-5-carboxylic acid (1b):
Brown crystals, m.p: 290-293 °C, IR (KBr, cm$^{-1}$): N-Hstr (3379), OHstr (2600-3350), aromtic C-Hstr ( 3040), aliphatic C-Hstr (2966, 2806), C=Ostr (1681), C=Nstr (1614), aromatic C=Cstr (1425-1573), C-Clstr (675), yield: 88%.

2-(chloromethyl)-5-nitro-1H-benzimidazole (1c):
dark yellow crystals, m.p:168-170 °C, IR (KBr, cm$^{-1}$): N-Hstr (3259), aromtic C-Hstr (3028), aliphatic C-Hstr (2980, 2800), C=Nstr (1647), NO$_2$str (1334, 1508), C-Clstr (690), yield: 85%.

General method for the Synthesis of 5-(un)substituted-2-mercapto-1H-benzimidazole 2(a,b) \(^{(16)}\)

A mixture of (0.05 mole) of 4-(un)substituted-o-phenylenediamine, ( 0.05 mole ) of carbon disulphide, (0.05 mole) of potassium hydroxide, (50 ml) of absolute ethanol and (10 ml) of water heated under reflux in 250 ml round bottom flask for 3 hours, then added cautiously (0.8 g) of charcoal and the reflux continued for 10 minutes, then charcoal was removed by filtration and (50 ml) of warm water added after heating the filtrate to 60-70 °C, and then acidified with dilute acidic acid with vigorous stirring, the precipitate was separated as a white crystals. The mixture obtained placed in ice path for 3 hours to complete the crystallization, the product obtained was filtrated, dried and recrystallized from ethanol, the completion of the reaction and the purity of the compounds were checked by TLC (mobile phase: hexane: ethyl acetate 1:2).

2-mercapto-1H-benzimidazole 2(a) :
White crystals, m.p: >300°C, IR (KBr, cm$^{-1}$): N-Hstr (3151), aromtic C-Hstr ( 3115), S-Hstr(2571) C=Nstr (1625), aromatic C=Cstr (1514, 1464), $^1$H-NMR (DMSO-d6) δ ppm: 1.8 (s, 1H, SH), 3.1 (s, 1H, NH), 7.5-7.79 (m, 4H, Ar-H), $^{13}$C-NMR (DMSO-d6) δ ppm: 109.3(C$_1$C$_2$), 122.2(C$_3$C$_6$), 132.2(C$_4$C$_5$), 168.0(C$_8$), Yield: 87%.

2-mercapto-5-methyl-1H-benzimidazole 2(b) :
Light brown crystals, m.p: 287-289°C, IR (KBr, cm$^{-1}$): N-H$_{str}$ (3126), aromatic C-H$_{str}$ (3041), aliphatic C-H$_{str}$ (2970-2862), S-H$_{str}$ (2571)  C=N$_{str}$ (1620), aromatic C=C$_{str}$ (1469-1571), $^{1}$H-NMR (DMSO-d$_{6}$) δ ppm: 1.93(s, 1H, SH), 3.0 (s, 3H, CH$_{3}$), 3.19 (s, 1H, NH), 7.6-7.8 (m, 3H, Ar-H), $^{13}$C-NMR (DMSO-d$_{6}$) δ ppm: 20.8(C$_{11}$), 109.0(C$_{3}$), 109.5(C$_{6}$), 122.9(C$_{2}$), 130.4(C$_{1}$), 131.3(C$_{4}$), 132.6(C$_{5}$), 167.7(C$_{8}$), Yield: 85%.

General method for synthesis of the compounds 3(a-f)$^{(17)}$

(0.42 g, 0.018 mole) of sodium was added to the solution of 5-(un)substituted-2-mercapto-1$^{H}$-benzimidazole 2(a,b) (0.016 mole) in methanol (30 ml) and then the mixture was strongly stirred for 20 minutes, 5-(un)substituted-2-(chloromethyl)-1$^{H}$-benzimidazole 1(a-c) (0.016 mole) was then added in 2-3 portions, and the resultant mixture was stirred for 2-8 hours. Precipitate appeared was filtered and washed with cold water or methanol and dried. The completion of the reaction and the purity of the compounds were checked by TLC (mobile phase: hexane: ethyl acetate 1:2).

2-(((1$^{H}$-benzo[d]imidazol-2- yl)methyl)thio)-1$^{H}$-benzo[d]imidazole 3a :

Pale yellow crystals, m.p: 272-275 °C, IR (KBr, cm$^{-1}$): N-H$_{str}$ (3387), aromatic C-H$_{str}$ (3053), aliphatic C-H$_{str}$ (2947,2885), C=N$_{str}$ (3155,1444), S-C$_{str}$ (740), $^{1}$H-NMR (DMSO-d$_{6}$) δ ppm: 3.88 (s, 2H, CH$_{2}$-), 4.79 (s, 2H, NH), 7.16-7.53 (m, 8H, Ar-H), $^{13}$C-NMR (DMSO-d$_{6}$) δ ppm: 28.8 (C$_{20}$), 121.5 (C$_{1},C_{2},C_{11},C_{12}$), 121.7 (C$_{3},C_{4},C_{13},C_{16}$), 149.2 (C$_{9},C_{5},C_{14},C_{15}$), 150.5(C$_{18}$), 153.1(C$_{8}$), Yield: 68%.

2-(((1$^{H}$-benzo[d]imidazol-2- yl)methyl)thio)-5-methyl-1$^{H}$-benzo[d]imidazole  3b :

Light yellow crystals, m.p: 207-208 dec. °C, IR (KBr, cm$^{-1}$): N-H$_{str}$ (3124), aromatic C-H$_{str}$ (3093), aliphatic C-H$_{str}$ (2968,2866), C=N$_{str}$ (1624), aromatic C=C$_{str}$ (1442-1519), S-C$_{str}$ (740), Yield: 70%.

2-(((1$^{H}$-benzo[d]imidazol-2- yl)thio)methyl)-1$^{H}$benzo[d]imidazole-5-carboxylic acid 3c :

Brown crystals, m.p: 190-192 °C, IR (KBr, cm$^{-1}$): N-H$_{str}$ (3143), aromatic C-H$_{str}$ (3057), aliphatic C-H$_{str}$ (2983,2899), C=N$_{str}$ (1624), aromatic C=C$_{str}$ (1531, 1415), S-C$_{str}$ (738), Yield: 45%.

2-(((5-methyl-1$^{H}$-benzo[d]imidazol- 2-yl)thio)methyl)-1$^{H}$benzo[d]imidazole-5-carboxylic acid 3d :

Brown crystals, m.p: >350 °C, IR (KBr, cm$^{-1}$): N-H$_{str}$ (3190), aromatic C-H$_{str}$ (3097), aliphatic C-H$_{str}$ (2993,2864), C=N$_{str}$ (1624), aromatic C=C$_{str}$ (1417-1529), S-C$_{str}$ (775), Yield: 56%.

2-(((1$^{H}$-benzo[d]imidazol-2- yl)thio)methyl)-5-nitro-1$^{H}$benzo[d]imidazole  3e :

Light yellow crystals, m.p: 230-232 °C, IR (KBr, cm$^{-1}$): N-H$_{str}$ (3190), aromatic C-H$_{str}$ (3095), aliphatic C-H$_{str}$ (2987,2906), C=N$_{str}$ (1625), aromatic C=C$_{str}$ (1440,1469), S-C$_{str}$ (740), NO$_{2}$$_{str}$ (1340.1517), $^{1}$H-NMR (DMSO-d$_{6}$) δ ppm: 3.73 (s, 2H, CH$_{2}$-), 4.85 (s, 2H, NH), 7.15-8.55 (m, 7H, Ar-H), Yield: 63%.

2-(((1$^{H}$-benzo[d]imidazol-2- yl)thio)methyl)-5-nitro-1$^{H}$benzo[d]imidazole  3f :

Light yellow crystals, m.p: 230-232 °C, IR (KBr, cm$^{-1}$): N-H$_{str}$ (3190), aromatic C-H$_{str}$ (3095), aliphatic C-H$_{str}$ (2987,2906), C=N$_{str}$ (1625), aromatic C=C$_{str}$ (1440,1469), S-C$_{str}$ (740), NO$_{2}$$_{str}$ (1340.1517), $^{1}$H-NMR (DMSO-d$_{6}$) δ ppm: 3.73 (s, 2H, CH$_{2}$-), 4.85 (s, 2H, NH), 7.15-8.55 (m, 7H, Ar-H), Yield: 63%.
5-methyl-2-((5-nitro-1Hbenzo[d]imidazol-2-yl)methyl)thio)-1H-benzo[d]imidazole 3f:
Dark yellow crystals, m.p: >350°C, IR (KBr, cm⁻¹): N-H str (3585), aromatic C-H str (3095), aliphatic C-H str (2970, 2866), C=N str (1627), aromatic C=C str (1442-1595), S-C str (736), NO₂ str (1342, 1521), Yield: 47%.

Result and discussion

The target compounds and the reactions sequence illustrated in scheme 1. The starting materials 1(a-c) 5-substituted-2-(chloromethyl)-1H-benimidazole were prepared depending on reported procedure by reacting 4-(un)substituted-o-phenylenediamine and chloroacetic acid, (14, 15, 18) the Structures of the compounds 1(a-c) were proved by comparison of its spectral data and physical properties with the reported projects. (15, 18, 19) 5-(un)substituted-2-mercapto-1H-benzimidazole 2(a,b) were also prepared from the reaction of 4-(un)substituted-o-phenylenediamine with carbon disulphide (CS₂) in the presence of potassium hydroxide in ethanol. The reaction sequence goes farther as showed in scheme 1 in order to yield different derivatives of bis benzimidazole 3(a-f) by the nucleophilic substitution reaction of the compounds 1(a-c) with the compounds 2(a,b) in the presence of sodium in methanol, the structures of the target compounds 3(a-f) were proved by FT-IR, ¹H-NMR, and ¹³C-NMR. All of the FT-IR spectra of the compound 3(a-f) revealed the disappearance of S-H stretching band which is at about (2579 cm⁻¹) and the appearance of C-S stretching band at the region (740-746 cm⁻¹), FT-IR spectrum of the compound 3a showed various adsorption band starting from Specific band at about (3387 cm⁻¹) related to benzimidazole N-H , (3053 cm⁻¹) for aromatic C-H , (2947, 2885 cm⁻¹) for aliphatic C-H stretching, (1620 cm⁻¹) for C=N stretching, and specific band at (740 cm⁻¹) related to C-S stretching. ¹H-NMR spectra showed the following chemical shifts starting from singlet signal at δ 3.88 related to methylene (CH₂-) proton , singlet signal also at δ 4.79 for benzimidazole N-H poroton, and multiplet signals at around δ 7.16-7.53 corresponded to benzene ring protons, while the ¹³C-NMR spectrum of the compound 3a was found
in equivalent with its assigned structure and showed these signals in δ ppm: 28.8, 121.5, 121.7, 149.2, 150.5, 153.1. The purity of the synthesized compounds was checked by TLC. Physical properties of the synthesized compounds are mentioned in Table 1.

Table 1: Physical properties of the synthesized compounds

| Comp no. | R     | R₁    | m.p (°C) | M.wt (g/mole) | Mol.Formula       | Color      | Yield % |
|----------|-------|-------|----------|---------------|-------------------|------------|---------|
| 1a       | H     |       | 147-150  | 166.61        | C₁₅H₁₂ClN₂       | yellow     | 93%     |
| 1b       | COOH  |       | 290-293  | 210.62        | C₁₅H₁₀ClN₂O₂     | brown      | 88%     |
| 1c       | NO₂   |       | 168-170  | 211.61        | C₁₅H₁₀ClN₂O₂     | dark yellow| 85%     |
| 2a       | H     |       | >300     | 150           | C₁₅H₁₀ClN₂S      | White      | 87%     |
| 2b       | CH₃   |       | 287-289  | 164           | C₁₆H₁₂N₂S        | Light brown| 85%     |
| 3a       | H     | H     | 272-275  | 280.3         | C₁₆H₁₂N₂S        | Pale yellow| 68%     |
| 3b       | H     | CH₃   | 207-208 dec. | 294 | C₁₆H₁₂N₂S | Light yellow | 70%     |
| 3c       | COOH  | H     | 190-192  | 324.3         | C₁₆H₁₀N₂O₂S      | brown      | 45%     |
| 3d       | COOH  | CH₃   | >350     | 338.3         | C₁₆H₁₀N₂O₂S      | brown      | 56%     |
| 3e       | NO₂   | H     | 230-232  | 325.3         | C₁₅H₁₁N₂O₂S      | Light yellow| 63%     |
| 3f       | NO₂   | CH₃   | >350     | 339           | C₁₅H₁₁N₂O₂S      | Dark yellow| 47%     |

dec.: decomposed

**Antibacterial activity**

most of the target compounds were tested for their in-vitro antibacterial activities toward two types of gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*) and two types of gram-positive bacteria (*Staphylococcus aureus*, *bacillus subtilis*) by using disc diffusion method, the concentration of the compounds were (10 mg/ml and 100 mg/ml). Inhibition zones were measured in millimeters and were compared with (ciprofloxacin and Ampicillin) as a standard antibiotics references. The results of the antibacterial activity tests are showed in (Table 2) which shows that all of the tested compounds for their antibacterial activity revealed good to moderate activity against *bacillus subtilis* bacteria whereas some of the tested compounds showed various activity against rest of the bacteria strains.

Table 2: antibacterial activity of the synthesized compounds

| Comp no. | Concentration (mg / ml) | Zone of inhibition ( in mm) |
|----------|-------------------------|-----------------------------|
|          |                         | Gram-positive | Gram-negative |
|          |                         | S. aureus | B. subtilis | P. aeruginosa | E. coli |
| 3a       | 10                      | 12  | 11  | -        | 12 |
| 3a       | 100                     | 12  | -   | -        | -   |
| 3c       | 10                      | 11  | 12  | 12       | 12 |
| 3c       | 100                     | -   | 12  | 12       | -   |
| 3d       | 10                      | -   | 11  | 11       | 15 |
| 3d       | 100                     | -   | 12  | 20       | -   |
| 3e       | 10                      | -   | 13  | -        | -   |
| 3e       | 100                     | 15  | 20  | 15       | 16 |
| 3f       | 10                      | 11  | 18  | -        | -   |
| 3f       | 100                     | 14  | 13  | -        | 15 |
| Ampicillin | 22                   | 23  | -   | -        | 10 |
| ciprofloxacin | 19               | 23  | 29  | -        | -   |
| DMSO solvent | 0                | 0   | 0   | 0        | 0   |
Conclusion

Different bis benzimidazole derivatives were synthesized through nucleophilic substitution reaction between 5-substituted-2-(chloromethyl)-1H-benzimidazole and 5-(un)substituted-2-mercapto-1H-benzimidazole. Structures of the compounds were confirmed by spectral methods (FT-IR, 1H-NMR, and 13C-NMR). The pharmacological study was performed to study the substituent effects on the antibacterial activity, some of the derivatives showed good to moderate activity against gram-negative (E.coli, P. aeruginosa) and gram-positive (B. subtilis, S. aureus) bacteria.

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