Synthesis and characterization of new compounds derived from 2-hydrazinobenzothiazole and evaluated their antibacterial activity

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Abstract: A series of new benzothiazole hydrazide derivatives starting from 2-mercaptobenzothiazole have been synthesized. 2-hydrazinobenzothiazole [M1] was prepared by refluxing of a mixture of hydrazine hydrate, H2O (80%) with 2-mercaptobenzothiazole. The reaction of the compound [M1] with the synthesized esters and amino esters [M2-M7 afforded benzothiazole hydrazide derivatives [M8-M13]. Compounds [M2-M7] were prepared from the reaction of different amino acids and carboxylic acids with absolute ethanol in presence of concentrated sulfuric acid. The structures of all synthesized compounds were established on the basis of FT-IR and some of them by 1H-NMR. The synthesized compounds have been evaluated for antimicrobial activity against Gram-positive and Gram-negative bacteria. Among thirteen synthesized compounds, in which four compounds (M8-M12) exhibited promising antibacterial activity.

Keywords: 2-hydrazinobenzothiazole, Amino acid, Carboxylic acid, hydrazine hydrate, biological activity.

1. Introduction

2-Hydrazine benzothiazole is a bicyclic ring system [1,2] bonded to the hydrazide group. Benzothiazole derivatives have been studied overall and take much interest. Its consider a core structure for the synthesis of new benzothiazole derivatives [3], this due to their several chemical reactivities and the potent broad spectrum of biological activity [4,5,6] such as anti-nociceptive [7], antitumor agents [8], antimicrobial [9], anti-inflammatory [10], anticancer [11,12], antibacterial [13,14], antifungal [15] and anti-HIV [16] and effective animal growth stimulators [17]. Furthermore, many of the benzothiazole derivatives and their metal complexes showed significant antibacterial and antifungal activity [18]. It was found that the action of many drugs increasing when managed as metal complexes than are free ligands [19].

2. Experimental:

2.1. Materials and measurements

2.1.1. Chemicals: All the chemicals and solvents used in this study were purchased from commercial sources (Merck, Fluka, and BDH-Chemical) Companies.
2.1.2. *Instruments:* The 1H-NMR spectra were recorded using CDCl3 and (DMSO-D6) as a solvent and TMS as internal standard at 298 K on Bruker 400 MHz Ultra-shied TM FT-NMR spectrometry in the laboratories of Al-Bayt University, Chemistry Department, Jordan. Chemical shifts are in parts per million (ppm).

2.1.3. *Microbial test:* All equipment required and strains of bacteria *S. aureus* and *E. Coli* and DMSO (solvent) were supplied from the Baghdad University, College of Science for Women, Biology Department.

2.2. *Preparation of compounds [1-13]:*

2.2.1. **Synthesis of Benzothiazole-2-yl-hydrazine [M1] [20]:** A mixture of 2-Mercaptobenzothiazole (0.0119 moles, 2 gram) and (8 ml) of hydrazine hydrate H₂O (80%) was refluxed for 4 hours. The mixture cooled at room temperature, then (5 ml) of ethanol was added. The separated precipitate was filtered and washed with cold water. The green crystals obtained, P. yield 92%, M.P. (202 °C).

2.2.2. **Synthesis of ester derivatives [M2-M7]:** A mixture of (1 mole) of various carboxylic acids dissolved in (5 ml) of absolute ethanol and (2.5 ml) of the concentrated sulfuric acid, was refluxed for 4 hours. After the reaction was completed it neutralized by addition of bicarbonate, then (5 ml) of ether was added until the precipitate formed. The precipitate was filtered and dried. The physical properties of compounds [M2-M7] respectively: P. yield %: 85, 86, 64, 89, 77, and 78. Color: yellow, pink, black, white, orange, and white crystals respectively, Melting point: (112-114°C), (96-98°C), (113-115°C), (124-126°C), (133-135°C) and (90-92°C) respectively.

2.2.3. **Synthesis of benzothiazole hydrazide (amide) derivatives compounds [M8-M13]:** 1mole or 2 moles) of ester derivatives [M2-M7] was mixed with (1 mole) of 2-hydrazinobenzothiazole [M1] in (5ml) of absolute ethanol. The mixture was refluxed for 4 hours, cooled by ice-bath, the precipitate was filtered and recrystallized by ethanol. The physical properties of compounds [M8-M13] respectively P. yields %: 77, 74, 81, 75, 67 and 83, Color: orange, yellow, pale black, green, black, and white. M.P.: (150-152°C), (144-146°C), (205-207°C), (187-189°C), (109-111°C) and (178-180°C) respectively.

2.3. *Antimicrobial Activity test:* The synthesized derivatives [M2, M6, M8, and M12] have been screened in vitro for their antibacterial activity against gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*) using the disk diffusion method [21] and DMSO as a solvent. The bacterial strains incubated at 37 °C for 24 hours. A standard 6mm diameter sterilizing filter paper impregnates with the above compounds were placed on agar with the tested microorganisms. The concentration of the tested compounds used was: 50, 100, 150, and 200 µL. The inhibition zone was measured in mm. The obtained results are listed in Tables 2a and 2b.

3. **Result and Discussion:**

The present work included the synthesis of new benzothiazole hydrazide derivatives, 2-hydrazinobenzothiazole prepared from the reaction of 2-mercaptobenzothiazole (MBT) with hydrazine hydrate under refluxing conditions. The synthesis of the desire compounds was accomplished according to the representation in scheme 1.
Scheme 1. The synthesis steps of 2-Hydrizinobenzothiazole derivatives starting from 2-mercapto benzothiazole.

3.1. Preparation of compounds:

3.1.1. Synthesis of 2-hydrizinobenzothiazole [M1]: 2-hydrizinobenzothiazole [M1] was synthesized by the nucleophilic substitution reactions of (2-mercapto benzothiazole) with hydrazine hydrate 80% and hydrogen sulfide displacement as a byproduct [22]. The FT-IR spectrum of 2-hydrizinobenzothiazole [M1] figure 1 shown bands at 3342 as, 3232 sym cm⁻¹ for \( \nu(C=O) \) (NH₂ str.) [23] and absorptions at 3101 which characterized for \( \nu(C=O) \) (NH str.), strong absorption at 1590 cm⁻¹ for \( \nu(C=O) \) (N-H bending), absorption at 1622 cm⁻¹ was due to \( \nu(C=S) \) (C=N str.). The sharp bands at 1417 cm⁻¹ due to the \( \nu(C=S) \) (C=C str.), stretching band at 3062 cm⁻¹ refer to \( \nu(C-H) \) aromatic, and 711 cm⁻¹ for \( \nu(C-S) \) [24].

![Figure 1](image1.png)

Figure 1. FT-IR spectrum of the compound (M1).

3.1.2. Synthesis of Ester derivatives [M2-M7]: FT-IR of compounds [M2-M7] figures (2-7) shown stretching bands at (1680-1733) Cm⁻¹ due to \( \nu(C=O) \) (O=C-Ostr.) The evidence for formation of ester is the disappearance of a significant band as a broad which could be attributed to the stretching of OH in a carboxylic acid group. [25]. The FTIR absorption bands of synthesized compounds shown in Table (1a). The 1HNMR DMSO-d6 δ Spectra for compound [M2] figure 8 showed the following data: 1.27 ppm (t, 3H, CH₃), 2.10 ppm (s, 1H, CH₃), 2.5-2.7 ppm (q, 2H, CH₂), 2.87 ppm (t, 2H, CH₂), 3.4 ppm (t, 1H, CH), 5.0-5.16 ppm (q, 2H, CH₂), 5.3 ppm (bs, 1H, NH₂). For compound [M6] figure 9: δ 1.26-1.28 ppm (t, 3H, CH₃), 3.2 ppm (t, 2H, CH₂), 4.1-4.4 ppm (q, 2H, CH₂), 5.3 ppm (bs, 2H, NH₂), 7.14-8.0 ppm (m, 4H, aromatic).
Figure 2. FT-IR spectrum of the compound (M2).

Figure 3. ¹H- NMR spectrum of the compound (M2).

Figure 4: FT-IR spectrum of the compound (M3).
Figure 5. FT-IR spectrum of the compound (M4).

Figure 6. FT-IR spectrum of the compound (M5).

Figure 7. FT-IR spectrum of the compound (M6).
3.2.3. Synthesis of Benzothiazole hydrazide derivatives compounds [M8-M13]: FT-IR for compounds [M8-M13] figures (10-15) were showed disappearance band of (C=O) carbonyl group of ester and appearance a new stratching band for (C=O) of amid group, appearance bands for (NHNH str.) group at about (31100-3200) Cm^{-1} Other bands shown in Table (1b). 1HNMR DMSO-d6 δ Spectra for compound [M10] figur 16 exhibits signals at: δ 2.83 ppm (bs, 1H, OH), 4.3 ppm (bs, 1H, NH), 4.6 ppm (s, 1H, CH), 7.5 ppm (s, 1H, NH), 7.8-8.6 ppm (m, 4H, aromatic). 1HNMR δ Spectra for compound [M11] figure 17 revealed the following signals: δ 2.4 ppm (t, 2H, CH₂), 4.2 ppm (bs, 1H, NH), 7.8 ppm (s, 1H, NH), 8.1-8.6 ppm (m, 4H, aromatic).
Figure 10. FT-IR spectrum of the compound (M8).

Figure 11. FT-IR spectrum of the compound (M9).

Figure 12. FT-IR spectrum of the compound (M10).
Figure 13. $^1$H-NMR spectrum of the compound (M10).

Figure 14. FT-IR spectrum of the compound (M11).

Figure 15. $^1$H-NMR spectrum of the compound (M11).
3.2. Antimicrobial activities:

The synthesized compounds [M2, M6, M8, and M12] were evaluated for their antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* bacteria's figure 18. The antibacterial activity of the synthesized compounds [M2, M6, M8, and M12] are summarized in Diagrams 1 and 2. The results reveals that compounds [M8 and M12] showed high inhibitory growth in the case of *E. coli* and *Staphylococcus aureus* in (150 and 200 µL), as compared to compounds (M2 and M6). The professional antibacterial activity of compounds M8 and M12 we can attribute may be to the presence of benzothiazole fragment in the structure of these compounds. Eventually, we can conclude that the compounds with benzothiazole hydrazide groups (M8 and M12) can inhibit the growth of the tested bacterial more than the compounds (M2 and M6).
Figure (18). Effect of compounds [M2, M6, M8 and M12] on *E. coli* and *S. aureus*.

Table 1a. Antibacterial activity of the synthesis compounds [M2, M6, M8 and M12] against E. coli.

| Compounds | 50 µg | 100 µg | 150 µg | 200 µg |
|-----------|-------|--------|--------|--------|
| M2        | +     | +      | +      | ++     |
| M6        | +     | +      | +      | ++     |
| M8        | -     | ++     | ++     | +++    |
| M12       | +     |        |        |        |

Table 1b. Antibacterial activity of the synthesis compounds [M2, M6, M8 and M12] against Staphylococcus aureus

| Compounds | 50 µg | 100 µg | 150 µg | 200 µg |
|-----------|-------|--------|--------|--------|
| M2        | +     | +      | +      | ++     |
| M6        | +     | +      | +      | ++     |
| M8        | +     | +      | ++     | +++    |
| M12       | +     | +      | ++     | +++    |

Key the symbols: (-) = no inhibition, (+) = (5-10) mm slightly active, (++) = (11-20) mm= moderately active, (+++) =more than 20mm= high active.

Diagram1: Antibacterial Activities (*Escherichia coli*) of Compounds [M2, M6, M8, M12],
Diagram2: Antibacterial Activities (*staphylococcus aureus*) of Compounds [M2, M6, M8, M12]
Table 2a. The FTIR spectrums of compounds [M2-M7].

| Structure | $\nu$C=O cm$^{-1}$ | $\nu$NH2 cm$^{-1}$ | $\nu$C-H cm$^{-1}$ | $\nu$C-N cm$^{-1}$ | $\nu$N-H cm$^{-1}$ | Other cm$^{-1}$ |
|-----------|-------------------|-------------------|-------------------|-------------------|-------------------|----------------|
| M2        | 1680              | 3342-3228assy. symm | 2824-2954         | 1076-1269         | ---               | C=O 1100-1220 |
|           |                   |                   |                   |                   |                   | C=O 1109-1176   |
|           |                   |                   |                   |                   |                   | C=O 1109-1176   |
|           |                   |                   |                   |                   |                   | C=O 1109-1176   |
| M3        | 1726.76           | guanine 3406-3332 | 2843-2858         | 1573-1597         | ---               | CO 1138-1230    |
|           |                   | NH2 (3255-3232)   |                   |                   |                   | NH rocking 882-941 |
|           |                   |                   |                   |                   |                   | NH rocking 882-941 |
|           |                   |                   |                   |                   |                   | NH rocking 882-941 |
| M4        | 1701              | 2844-2854         | 2854-2950         | 1573-1597         | ---               | CO 1138-1461    |
|           |                   |                   |                   |                   |                   | CH3 rocking 873-710 |
|           |                   |                   |                   |                   |                   | CH3 rocking 873-710 |
|           |                   |                   |                   |                   |                   | CH3 rocking 873-710 |
| M5        | 1706              | 2852-2950         | 1573-1597         | 1516-1548         | ---               | CO 1138-1461    |
|           |                   |                   |                   |                   |                   | CH3 rocking 873-710 |
|           |                   |                   |                   |                   |                   | CH3 rocking 873-710 |
| M6        | 1732              | 3340-3280         | 2854-2939         | 1516-1548         | 3170-3172        | CO 1161-1211    |
|           |                   |                   |                   |                   |                   | CHa 1020         |
|           |                   |                   |                   |                   |                   | CHa 1020         |
| M7        | 1733              | 2929-2979         | 1516-1548         | 3170-3172        | 3095-3095        | CO 1161-1211    |
|           |                   |                   |                   |                   |                   | CHa 1020         |
|           |                   |                   |                   |                   |                   | CHa 1020         |

Table 2b. The FTIR spectrums of compounds [M8-M13].

| Structure | $\nu$C=O cm$^{-1}$ | $\nu$C=C cm$^{-1}$ | $\nu$NH2 cm$^{-1}$ | $\nu$NH cm$^{-1}$ | $\nu$C-H arcm cm$^{-1}$ | $\nu$C-S cm$^{-1}$ | Other cm$^{-1}$ |
|-----------|-------------------|-------------------|-------------------|-------------------|-------------------------|------------------|----------------|
| M8        | 1639              | 1512              | 3261-3272         | 3201              | 3074                    | 628              | C=S 1564      |
|           |                   |                   |                   |                   |                         |                   | CH aliph. 2868-2954 |
|           |                   |                   |                   |                   |                         |                   | CH aliph. 2868-2954 |
|           |                   |                   |                   |                   |                         |                   | CH aliph. 2868-2954 |
| M9        | 1473              | 3375-3276         | 3166-3176         | 3028-3095         |                         |                   | C=S 1564      |
|           |                   |                   |                   |                   |                         |                   | CH aliph. 2850-2920 |
|           |                   |                   |                   |                   |                         |                   | CH aliph. 2850-2920 |
|           |                   |                   |                   |                   |                         |                   | CH aliph. 2850-2920 |
| M10       | 1647              | 1558              | 3174-3219         | 3024              |                         |                   | C=S 1561      |
|           |                   |                   |                   |                   |                         |                   | CH aliph. 2966-2873 |
|           |                   |                   |                   |                   |                         |                   | CH aliph. 2966-2873 |
|           |                   |                   |                   |                   |                         |                   | CH aliph. 2966-2873 |
|           |                   |                   |                   |                   |                         |                   | CH aliph. 2966-2873 |

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4. Conclusion:

In conclusion, we developed a convenient and high yielding methodology for the synthesis of novel 2-hydrazinobenzothiazole derivatives. All synthesized compounds were confirmed by FT-IR, and the structures of compounds [M2, M6, M12, M11, and M13] were established by $^1$H-NMR. Some of these compounds evaluated for in vitro antibacterial activity against E. coli (+ve gram) and S. aureus (-ve gram) strains and the results showed that compounds M8 and M12 possess a potent activity as compared to M2 and M6. The presence of benzothiazole fragment may be play a main role of their antimicrobial activity. This study may be e useful for the development of potential antibacterial candidates derived from benzothiazole hydrazide.

Acknowledgment:

We appreciate the support and encouragement of the chemistry department / College of Education – Iraqi University represented by the head of the department and all the staff. My gratitude is also express to the Ibn-Sina State / Ministry of Industry and Materials for the completion of the FT-IR spectra. Also, our appreciation goes to Al-albyt University to complete the $^1$H-NMR spectrum.

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